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(54) Title: COMPOSITIONS AND METHODS USING SAME FOR TREATING AMYLOID ASSOCIATED DISEASES

(57) Abstract: Indole derivatives, compositions including same, and methods utilizing same for the treatment of amyloid associated diseases, such as type II diabetes mellitus, Alzheimer's dementia or diseases, systemic and localized amyloidosis, and prion-related encephalopathies are provided.



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COMPOSITIONS AND METHODS USING SAME FOR TREATING AMYLOID ASSOCIATED DISEASES

FIELD AND BACKGROUND OF THE INVENTION

5 The present invention relates to compounds, compositions and methods using the same for preventing amyloid fibril formation. More particularly, the present invention relates to indole derivatives, compositions including same, and methods using the same, for the treatment of amyloid associated diseases, such as type II diabetes mellitus, Alzheimer's dementia or diseases, systemic and localized
10 amyloidosis, and prion-related encephalopathies.

Amyloid material deposition (also referred to as amyloid plaque formation) is a central feature of a variety of unrelated pathological conditions including Alzheimer's disease, prion-related encephalopathies, type II diabetes mellitus, familial amyloidosis, secondary amyloidosis, and light-chain amyloidosis.

15 Amyloid material is composed of a dense network of rigid, nonbranching proteinaceous fibrils of indefinite length that are about 80 to 100 Å in diameter. Amyloid fibrils contain a core structure of polypeptide chains arranged in antiparallel β -pleated sheets lying with their long axes perpendicular to the long axis of the fibril [Both et al. (1997) Nature 385:787-93; Glenner (1980) N. Eng. J. Med. 302:1283-92].

20 Approximately twenty amyloid fibril proteins have been identified in-vivo and correlated with specific diseases. These proteins share little or no amino acid sequence homology, however the core structure of the amyloid fibrils is essentially the same. This common core structure of amyloid fibrils and the presence of common substances in amyloid deposits suggest that data characterizing a particular form of
25 amyloid material may also be relevant to other forms of amyloid material and thus can be implemented in template design for the development of drugs for use against amyloid associated diseases such as type II diabetes mellitus, Alzheimer's dementia or diseases, and prion-related encephalopathies.

Furthermore, amyloid deposits do not appear to be inert in vivo, but rather are
30 in a dynamic state of turnover and can even regress if the formation of fibrils is halted [Gillmore et al. (1997) Br. J. Haematol. 99:245-56].

Thus, therapies designed to inhibiting the production of amyloid polypeptides or inhibiting amyloidosis may be useful for treating amyloid associated diseases.

Inhibition of the production of amyloid polypeptides: Direct inhibition of the production of amyloid polypeptides may be accomplished, for example, through the use of antisense oligonucleotides, such as against human islet amyloid polypeptide messenger RNA (mRNA). *hi vitro*, the addition of antisense oligonucleotides or the expression of antisense complementary DNA against islet amyloid polypeptide mRNA increases insulin mRNA and protein content of cells, demonstrating the potential effectiveness of this approach [Kulkarni et al. (1996) J. Endocrinol. 151:341-8; Novials et al. (1998) Pancreas 17:182-6]. However, no experimental result demonstrating the *in vivo* effectiveness of such antisense molecules has been disclosed.

Inhibition of the formation of amyloid fibrils: Amyloid, including islet amyloid, contains potential stabilizing or protective substances, such as serum amyloid P component, apolipoprotein E, and perlecan. Blocking their binding to developing amyloid fibrils could inhibit amyloidogenesis [Kahn et al. (1999) Diabetes 48:241-53], as could treatment with antibodies specific for certain parts of an amyloidogenic protein [Solomon et al. (1997) Proc. Natl. Acad. Sci USA 94:4109-12].

The following summarizes current attempts to engineer drugs having the capability of destabilizing amyloid structures.

Destabilizing compounds: Heparin sulfate has been identified as a component of all amyloids and has also been implicated in the earliest stages of inflammation associated amyloid induction. Kisilevsky and co-workers (Mature Med. 1:143-148, 1995) described the use of low molecular weight anionic sulfonate or sulfate compounds that interfere with the interaction of heparin sulfate with the inflammation associated amyloid precursor and the β peptide of Alzheimer's disease (AD). Heparin sulfate specifically influences the soluble amyloid precursor (SAA2) to adopt an increased β -sheet structure characteristic of the protein folding pattern of amyloids. These anionic sulfonate or sulfate compounds were shown to inhibit heparin accelerated A β fibril formation, and were able to disassemble preformed fibrils *in vitro*, as monitored by electron micrography. Moreover, these compounds substantially arrested murine splenic inflammation associated amyloid progression *in vivo* in acute and chronic models. However, the most potent compound [i.e., poly-(vinylsulfonate)] showed acute toxicity. Similar toxicity has been observed with

another compound, IDOX (Anthracycline 4'-iodo-4'-deoxy-doxorubicin), which has been observed to induce amyloid resorption in patients with immunoglobulin light chain amyloidosis (AL) [Merlini et al. (1995) Proc. Natl. Acad. Sci. USA].

Destabilizing antibodies: Anti- β -amyloid monoclonal antibodies have been shown to be effective in disaggregating β -amyloid plaques and preventing β -amyloid plaque formation *in vitro* (U.S. Pat. No. 5,688,561). However, no experimental result demonstrating the *in vivo* effectiveness of such antibodies has been disclosed.

Small molecules: The potential use of small molecules which bind amyloid polypeptide and stabilize the native fold of the protein has been attempted in the case of transthyretin (TTR) protein [Peterson (1998) Proc. Natl. Acad. Sci. USA 95:12965-12960; Oza (1999) Bioorg. Med. Chem. Lett. 9:1-6]. Thus far, it has been demonstrated that molecules such as thyroxine and fiufenamic acid are capable of preventing the conformation change, leading to amyloid formation. However, the use of such compounds in animal models has not yet been proven, and might be compromised due to the presence of such compounds in blood, or in proteins other than TTR, which are capable of binding these ligands.

Antioxidants: Another proposed therapy has been the intake of antioxidants in order to avoid oxidative stress and maintain amyloid proteins in their reduced state (i.e., monomers and dimers). The use of sulfite was shown to lead to more stable monomers of TTR protein both *in vitro* and *in vivo* [Altland (1999) Neurogenetics 2:183-188]. However, a complete characterization of the antioxidant effect is still not available and the interpretation of results concerning possible therapeutic strategies remains unclear.

Destabilizing peptides: The finding that the addition of synthetic peptides that disrupt the β -pleated sheets (' β -sheet breakers'), thereby dissociating fibrils and preventing amyloidosis [Soto et al. (1998) Nat. Med. 4:822-6,], is particularly promising from a clinical point of view. In that disclosure, it was found that a penta-residue peptide inhibited amyloid beta-protein fibrillogenesis, disassembled preformed fibrils *in vitro*, and prevented neuronal death induced by fibrils in cell culture. In addition, the beta-sheet breaker peptide significantly reduced amyloid beta-protein deposition *in vivo*, and completely blocked the formation of amyloid fibrils in a rat brain model of amyloidosis.

Green tea extracts: U.S. Patent Applications having the Publication Nos. 20020086067 and 20020151506 teach the use of various components of green tea extracts for treating an amyloid disease. While these patent applications teach that these components inhibit amyloid fibril formation, they fail to teach neither a
5 mechanism nor a common structural feature which provides these green tea components with such an activity. The present inventor has previously shown that aromatic interactions play a key role in amyloid fibril formation by serving as structural and functional elements that direct molecular recognition and self-assembly and have further shown that such aromatic interactions can be induced by tryptophane
10 containing peptide sequences [Gazit (2002) FASEB J. 16:77-83].

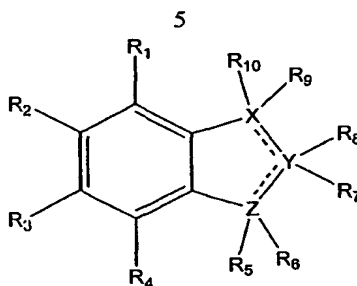
As is well known, tryptophane is an amino acid residue having an indole side-chain. Thus, while conceiving the present invention, it was envisioned that compounds having an aromatic moiety and particularly an indole moiety, which may participate in such aromatic interactions, could efficiently serve as inhibitors of
15 amyloid fibril formation. It was further envisioned that since indoles are known to act as antioxidants [Herraiz et al. (2004) Free Radic Res. 38:323-31], compounds having an indole moiety could serve simultaneously as inhibitors of amyloid fibril formation and as antioxidants and as such may exert synergistic effect in treating amyloid-associated diseases.

20

SUMMARY OF THE INVENTION

While reducing the present invention to practice, the present inventors indeed empirically determined that amyloid formation can be strongly inhibited by indole derivatives, such as indole-3-carbinol (3-hydroxymethyl indole), 3-hydroxyindole,
25 and 4-hydroxyindole, suggesting use of various indole compounds in the treatment of amyloid associated diseases.

Hence, according to one aspect of the present invention there is provided a method of treating an amyloid associated disease in a subject, the method comprising administering to a subject in need thereof, a therapeutically effective amount of a
30 compound having the general formula:



a pharmaceutically acceptable salt thereof, or a prodrug thereof,
wherein:

- 5 the dashed line denotes a double bond either between X and Y or between Y and Z;

X, Y and Z are each independently selected from the group consisting of carbon and nitrogen, whereas at least one of X, Y, and Z is nitrogen; and

- 10 R_1 - R_{10} are each independently selected from the group consisting of hydrogen, lone pair electrons, hydroxy, alkyl, cycloalkyl, phenyl, phenol, hydroxyphenol, dihydroxyphenol, aryl, alkenyl, alkynyl, heteroaryl, heteroalicyclic, halo, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, C-carboxy, O-carboxy, thiocarboxy, carbonyl, oxo, thiocarbonyl, sulfinyl, and sulfonyl, or absent, or, alternatively, at least two of R_1 - R_{10} form at least one five- or six-membered aromatic, heteroaromatic,
15 alicyclic or heteroalicyclic ring, thereby treating the amyloid associated disease in the subject.

According to further features in preferred embodiments of the invention described below, each of X and Y is carbon; and Z is nitrogen.

- 20 According to still further features in the described preferred embodiments, the double bond is between X and Y.

According to still further features in the described preferred embodiments at least one of R_1 - R_{10} comprises an electronegative group.

According to still further features in the described preferred embodiments at least one of R_1 and R_9 comprises an electronegative group.

The electronegative group can be, for example, hydroxy or halo (e.g., chloro, bromo and iodo).

According to still further features in the described preferred embodiments, at least one of R_1 - R_{10} comprises a hydroxy group.

According to still further features in the described preferred embodiments, the double bond is between X and Y and at least one of R_1 - R_{10} comprises a hydroxy group.

According to still further features in the described preferred embodiments, at least one Of R_1 and R_9 comprises a hydroxy group.

According to still further features in the described preferred embodiments, at least one Of R_1 and R_9 is a hydroxy group.

According to still further features in the described preferred embodiments, each Of R_2 - R_5 and R_7 is hydrogen and R_6 , R_8 and R_{10} are absent, the compound being either 3-hydroxyindole or 4-hydroxyindole.

According to still further features in the described preferred embodiments, R_1 is hydrogen and R_9 is a hydroxy group, the compound being 3-hydroxyindole.

According to still further features in the described preferred embodiments, R_1 is a hydroxy group and R_9 is hydrogen, the compound being 4-hydroxyindole.

According to still further features in the described preferred embodiments, at least one Of R_1 - R_{10} is a hydroxyalkyl.

According to still further features in the described preferred embodiments, at least one of R_7 and R_9 is a hydroxyalkyl.

According to still further features in the described preferred embodiments, each Of R_1 - R_5 is hydrogen, and R_6 , R_8 and R_{10} are absent.

According to still further features in the described preferred embodiments, the hydroxyalkyl is hydroxymethyl.

According to still further features in the described preferred embodiments, R_7 is hydrogen and R_9 is hydroxymethyl, the compound being indole-3-carbinol (3-hydroxymethyl indole).

According to still further features in the described preferred embodiments, each R_7 and R_9 is hydroxyalkyl, the compound being a 2,3-dihydroxyalkyl indole.

According to still further features in the described preferred embodiments, the administering is effected at a concentration of the compound not exceeding 4 mg/kg body weight/hour.

According to still further features in the described preferred embodiments, the administering is effected orally.

According to another aspect of the present invention, there is provided use of the compound described hereinabove, for the manufacture of a medicament identified for the treatment of amyloid associated diseases.

According to another aspect of the present invention there is provided a pharmaceutical composition comprising a therapeutically effective amount of the compound described hereinabove and a pharmaceutically acceptable carrier.

According to further features in the described preferred embodiments, the pharmaceutical composition further comprises an anti-amyloid drug.

According to still further features in the described preferred embodiments, the anti-amyloid drug is selected from the group consisting of an amyloid destabilizing antibody, an amyloid destabilizing peptide and an anti-amyloid small molecule.

According to another aspect of the present invention there is provided an article-of-manufacture comprising a packaging material and a pharmaceutical composition identified for treating amyloid associated diseases being contained within the packaging material, the pharmaceutical composition including, as an active ingredient, the compound described hereinabove, and a pharmaceutically acceptable carrier.

According to another aspect of the present invention there is provided a compound having the above described general formula, with the proviso that the compound is not any one selected from the group consisting of indole, 2-hydroxyindole, 3-hydroxyindole, 4-hydroxyindole, 5-hydroxyindole, 3-methyl indole, indole-3-acetic acid, indole-3-butyric acid, indole-3-carboxylic acid, indole-3-propionic acid, indole-3-ethanol, indole-3-carbinol, and a 2,3-hydroxyalkyl indole.

According to further features in the described preferred embodiments of the compound of the present invention, each of X and Y is carbon; and Z is nitrogen.

According to further features in the described preferred embodiments of the compound of the present invention, the double bond is between X and Y.

According to further features in the described preferred embodiments of the compound of the present invention, at least one $\text{OfR}_1\text{-R}_{10}$ comprises a hydroxy group.

According to further features in the described preferred embodiments of the compound of the present invention, at least one $\text{OfR}_1\text{-R}_{10}$ is a hydroxyalkyl.

The present invention successfully addresses the shortcomings of the presently known configurations by providing novel compounds and compositions which can be

efficiently used for preventing amyloid fibril formation and thus for treating amyloid associated diseases.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

FIGs. 1a-c present the 2D chemical structures of indole-3-carbinol (3-hydroxymethyl indole), 3-hydroxyindole, and 4-hydroxyindole, as exemplary indole derivatives which can be used for implementing the present invention;

FIG. 2 presents comparative plots illustrating inhibition of β -amyloid fibril formation by indole-3-carbinol, as observed by monitoring fluorescence measurements during a Thioflavin T (ThT) fluorescence binding assay of A β 1-40 polypeptide samples in the absence (squares) and presence (triangles) of the indole-3-carbinol, in accordance with the present embodiments;

FIGs. 3a-b present electron micrographs showing microstructural features of representative fluorescence assay samples of A β 1-40 (per Figure 2) in the absence (Figure 3a) and presence (Figure 3b) of indole-3-carbinol, after attainment of the

plateau at about 192 hours, and clearly demonstrating inhibition of β -amyloid fibril formation by the indole-3-carbinol, in accordance with the present embodiments;

FIG. 4 presents a comparative bar graph illustrating the inhibition of β -amyloid fibril formation as effected by indole-3-carbinol (denoted as IC), 3-hydroxyindole (denoted as 3HI), and 4-hydroxyindole (denoted as 4HI), indole-3-acetic acid (denoted as IAA), indole-3-butyric acid (denoted as IBA), indole-3-carboxylic acid (denoted as I3CA), 2-hydroxyindole (denoted as 2HI), 5-hydroxyindole (denoted as 5HI), 3-methyl indole (denoted as 3MI), indole-3-propionic acid (denoted as IP), indole-3-ethanol (IE) and (denoted as IE)], unmodified indole (denoted as IND), and the control [(denoted as WT) - absent of any indole species], individually, as observed by monitoring fluorescence measurements during a Thioflavin T (ThT) fluorescence binding assay of $A\beta$ 1-42 samples, in accordance with the present embodiments;

FIG. 5 presents comparative plots illustrating the inhibition of β -amyloid fibril formation as effected by various concentrations indole-3-carbinol (denoted as I3C, rhombus), 3-hydroxyindole (denoted as 3HI, squares), and 4-hydroxyindole (denoted as 4HI, triangles), individually, as observed by monitoring fluorescence measurements during a Thioflavin T (ThT) fluorescence binding assay of $A\beta$ 1-42 samples, in accordance with the present embodiments;

FIGS. 6a-c present comparative plots illustrating the kinetics of β -amyloid fibril formation following 14 days incubation of a fixed 5 μ M concentration of $A\beta$ 1-40 with 5 μ M (triangles), 25 μ M (squares) and 50 μ M (stars) of 4-hydroxyindole (denoted as 4HI, Figure 6a), 3-hydroxyindole (denoted as 3HI, Figure 6b), and indole-3-carbinol (denoted as I3C, Figure 6c), or without an indole derivative (control, triangles), as observed by monitoring fluorescence measurements during a Thioflavin T (ThT) fluorescence binding assay of $A\beta$ 1-40 samples, in accordance with the present embodiments;

FIGS. 7a-d present electron micrographs (scale bar represents 200 nm) showing microstructural features of representative fluorescence assay samples of $A\beta$ 1-40 (incubated for 14 days, per Figure 5) in the absence (Figure 7a) and presence of 25 μ M 4-hydroxyindole (Figure 7b), 50 μ M 3-hydroxyindole (Figure 7c), and 50 μ M indole-3-carbinol (Figure 7d);

FIGs. 8a-d present AFM height images (5 μm x 5 μm) showing structural features of representative fluorescence assay samples of A β 1-40 (per Figure 5, taken once the control samples reached the polymerization phase), deposited on mica following incubation in the absence (control, Figure 8a) and presence of 50 μM 4-hydroxyindole (Figure 8b), 50 μM 3-hydroxyindole (Figure 8c), and 50 μM indole-3-carbinol (Figure 9d);

FIGs. 9a-b present bar graphs demonstrating the effect of a solution containing A β 1-40 in the absence (control) and presence of various concentrations of indole-3-carbinol (denoted I3C), 3-hydroxyindole (denoted 3HI) and 4-hydroxyindole (denoted 4-HI), immediately following its preparation (Figure 9a) and following 7 days incubation (Figure 9b) on the viability of PC12 cells; and

FIG. 10 presents a bar graph showing the effect of a solution containing A β 1-40 in the absence (control) and presence of various concentrations of 4-hydroxyindole (denoted 4-HI), following 7 days incubation (Figure 9b), on the viability of PC12 cells.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of indole derivatives and compositions including same, which can be efficiently used for preventing amyloid fibril formation and thus for the treatment of amyloid associated diseases, such as type II diabetes mellitus, Alzheimer's dementia or diseases, and prion-related encephalopathies.

The principles and operation of the compounds, compositions and methods according to the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

Numerous therapeutic approaches for prevention of amyloid fibril formation or disaggregation of amyloid material have been described in the prior art. However,

current therapeutic approaches are limited by cytotoxicity, non-specificity and delivery barriers.

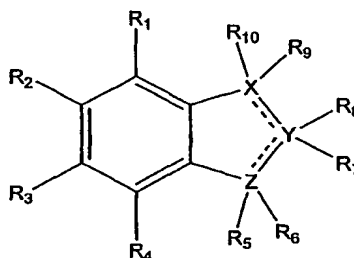
The present inventors have previously shown that aromatic interactions play a key role in amyloid fibril formation by serving as structural and functional elements that direct molecular recognition and self-assembly [Azriel and Gazit (2001) *J. Biol. Chem* 276: 34156-34161; Gazit (2002) *FASEBJ.* 16:77-83]. Consequently, aromatic peptides and, particularly aromatic peptides that include one or more tryptophane residues, were shown to inhibit amyloid fibril formation.

It was therefore envisioned that compounds having an indole moiety, as in tryptophane, can participate in the aromatic interactions described above and would thus inhibit amyloid formation.

While reducing the present invention to practice, the present inventors empirically determined that amyloid formation can be strongly inhibited by indole derivatives, such as indole-3-carbinol (3-hydroxymethyl indole), 3-hydroxyindole, and 4-hydroxyindole, (see, Figure 1), suggesting use of these compounds in the treatment of amyloid associated diseases.

Indole-3-carbinol is known as a food supplement that can be used in anti-cancer therapy [Donald et al. (2004) *Int J Cancer.* 111:961-7]. However, the use of indole-3-carbinol, or other indole derivatives, such as 3-hydroxyindole, and 4-hydroxyindole, for inhibiting formation of amyloid fibrils has never been suggested nor practiced hitherto.

An indole derivative, according to the present invention, is represented by the general formula:



a pharmaceutically acceptable salt thereof, or a prodrug thereof, wherein:

the dashed line denotes a double bond either between X and Y, or, between Y and Z;

X, Y and Z are each independently selected from the group consisting of carbon and nitrogen, whereas at least one of X, Y, and Z is nitrogen; and

5 Ri-Ri₀ are each independently selected from the group consisting of hydrogen, lone pair electrons, hydroxy, alkyl, cycloalkyl, phenyl, phenol, hydroxyphenol, dihydroxyphenol, aryl, alkenyl, alkynyl, heteroaryl, heteroalicyclic, halo, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, C-carboxy, O-carboxy, thiocarboxy, carbonyl, oxo, thiocarbonyl, sulfinyl, and sulfonyl, or absent, or, alternatively, at least
10 two of Ri-Ri₀ form at least one five- or six-membered aromatic, heteroaromatic, alicyclic or heteroalicyclic ring.

As used herein, the term 'alkyl' refers to a saturated aliphatic hydrocarbon including straight chain and branched chain groups. Preferably, the alkyl group has 1 to 20 carbon atoms. Whenever a numerical range; *e.g.*, '1-20', is stated herein, it
15 implies that the group, in this case the alkyl group, may contain 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc., up to and including 20 carbon atoms. More preferably, the alkyl is a medium size alkyl having 1 to 10 carbon atoms. Most preferably, unless otherwise indicated, the alkyl is a lower alkyl having 1 to 4 carbon atoms. The alkyl group may be substituted or unsubstituted. When substituted, the
20 substituent group can be, for example, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphonium, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea,
25 thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, and amino, as these terms are defined hereinbelow.

As used herein, the term 'hydroxyalkyl' refers to an alkyl, as defined hereinabove, which is further substituted by one or more hydroxy groups. In the
30 compound including two or more carbon atoms and two or more hydroxy groups, then each hydroxy group can be on the same carbon atom or on different carbon atoms. The hydroxyalkyl may be additionally substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, hydroxyalkyl, trihaloalkyl,

cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphonium, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, and amino, as these terms are defined herein.

A 'cycloalkyl' group refers to an all-carbon monocyclic or fused ring (*Le.*, rings which share an adjacent pair of carbon atoms) group wherein one of more of the rings does not have a completely conjugated pi-electron system. Examples, without limitation, of cycloalkyl groups are cyclopropane, cyclobutane, cyclopentane, cyclopentene, cyclohexane, cyclohexadiene, cycloheptane, cycloheptatriene, and adamantane. A cycloalkyl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphonium, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, and amino, as these terms are defined hereinbelow.

A 'hydroxy' group refers to an -OH group.

An 'alkenyl' group refers to an alkyl group, as defined hereinabove, which consists of at least two carbon atoms and at least one carbon-carbon double bond.

An 'alkynyl' group refers to an alkyl group, as defined hereinabove, which consists of at least two carbon atoms and at least one carbon-carbon triple bond.

An 'aryl' group refers to an all-carbon monocyclic or fused-ring polycyclic (*i.e.*, rings which share adjacent pairs of carbon atoms) groups having a completely conjugated pi-electron system. Examples, without limitation, of aryl groups are phenyl, naphthalenyl and anthracenyl. The aryl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl,

heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfmyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphonium, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, and amino, as these terms are defined herein.

As used herein, the term 'phenol' refers to a phenyl substituted by an hydroxy group. The phenol group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphonium, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, and amino, as these terms are defined herein.

As used herein, the term 'hydroxyphenol' which also encompasses the term 'dihydroxyphenol' refers to a phenol, as defined hereinabove, which is further substituted by one or more additional hydroxy groups. The additional hydroxy groups can be at the *para*, *ortho* and/or *meta* positions with respect to the hydroxy group of the phenol. The hydroxyphenol may be additionally substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphonium, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, and amino, as these terms are defined herein.

A 'heteroaryl' group refers to a monocyclic or fused ring (*i.e.*, rings which share an adjacent pair of atoms) group having in the ring(s) one or more hetero-atoms,

such as, for example, nitrogen, oxygen and sulfur and, in addition, having a completely conjugated pi-electron system. Examples, without limitation, of heteroaryl groups include pyrrole, furane, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrimidine, quinoline, isoquinoline and purine. The heteroaryl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphonium, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, and amino, as these terms are defined herein.

A 'heteroalicyclic' group refers to a monocyclic or fused ring group having in the ring(s) one or more hetero-atoms such as nitrogen, oxygen and sulfur. The rings may also have one or more double bonds. However, the rings do not have a completely conjugated pi-electron system. The heteroalicyclic may be substituted or unsubstituted. When substituted, the substituted group can be, for example, lone pair electrons, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphonium, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, and amino, as these terms are defined herein. Representative examples are piperidine, piperazine, tetrahydrofuran, tetrahydropyran, morpholino and the like.

A 'halo' group refers to fluoro, chloro, bromo or iodo.

An 'alkoxy' group refers to both an -O-alkyl and an -O-cycloalkyl group, as defined herein.

An 'aryloxy' group refers to both an -O-aryl and an -O-heteroaryl group, as defined herein.

A 'thiohydroxy' group refers to a -SH group.

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A 'thioalkoxy' group refers to both an -S-alkyl group, and an -S-cycloalkyl group, as defined herein.

A 'thioaryloxy' group refers to both an -S-aryl and an -S-heteroaryl group, as defined herein.

5 An 'oxo' group refers to an =O group.

A 'carbonyl' group refers to a -C(=O)-R' group, where R' is hydrogen, alkyl, alkenyl, cycloalkyl, aryl, heteroaryl (bonded through a ring carbon) or heteroalicyclic (bonded through a ring carbon) as defined herein.

10 A 'thiocarbonyl' group refers to a -C(=S)-R' group, where R' is as defined herein for R'.

A 'C-carboxy' group refers to a -C(=O)-O-R' groups, where R' is as defined herein.

An 'O-carboxy' group refers to a R'C(=O)-O- group, where R' is as defined herein.

15 A 'thiocarboxy' group refers to a R'C(^O)-S- group, where R' is as defined herein.

A 'sulfinyl' group refers to an -S(=O)-R' group, where R' is as defined herein.

A 'sulfonyl' group refers to an -S(=O)₂-R' group, where R' is as defined herein.

20 A 'trihalomethyl' group refers to a -CX group wherein X is a halo group as defined herein.

A 'trihalomethanesulfonyl' group refers to a X₃CS(=O)₂- group wherein X is a halo group as defined herein.

A 'S-sulfonamido' group refers to a -S(=O)₂-NR'R" group, with R' and R" as defined herein.

25 A 'N-sulfonamido' group refers to an R'S(=O)₂-NR" group, where R' and R" are as defined herein.

A 'trihalomethanesulfonamido' group refers to an X₃CS(=O)₂NR'- group, where R' and X are as defined herein.

30 An 'O-carbamyl' group refers to an -OC(=O)-NR'R" group, where R' and R" are as defined herein.

A 'N-carbamyl' group refers to an R"OC(=O)-NR'- group, where R' and R" are as defined herein.

An 'O-thiocarbamyl' group refers to an $-\text{OC}(=\text{S})-\text{NR}'\text{R}''$ group, where R and R'' are as defined herein.

A 'N-thiocarbamyl' group refers to an $\text{R}''\text{OC}(=\text{S})\text{NR}'-$ group, where R' and R'' are as defined herein.

5 An 'amino' group refers to an $-\text{NR}'\text{R}''$ group where R' and R'' are as defined herein.

A 'C-amido' group refers to a $-\text{C}(=\text{O})-\text{NR}'\text{R}''$ group, where R and R'' are as defined herein.

10 A 'N-amido' group refers to an $\text{R}'\text{C}(=\text{O})-\text{NR}''$ group, where R' and R'' are as defined herein.

An 'urea' group refers to an $-\text{NR}'\text{C}(=\text{O})-\text{NR}''\text{R}'''$ group, where R' and R'' are as defined herein and R''' is defined as either R' or R''.

A 'guanidino' group refers to an $-\text{R}'\text{NCC}=\text{N}-\text{NR}''\text{R}'''$ group, where R', R'' and R''' are as defined herein.

15 A 'guanyl' group refers to an $\text{R}'\text{R}''\text{NC}(=\text{N})-$ group, where R and R'' are as defined herein.

A 'nitro' group refers to an $-\text{NO}_2$ group.

A 'cyano' group refers to a $-\text{G}\equiv\text{N}$ group.

An 'azo' group refers to a $-\text{N}=\text{N}$ group.

20 The term 'phosphonyl' describes a $-\text{O}-\text{P}(=\text{O})(\text{OR}')-$ group, with R' as defined hereinabove.

The term 'phosphinyl' describes a $-\text{PR}'-$ group, with R' as defined hereinabove.

The term 'phosphonium' is a $-\text{P}^+\text{R}'\text{R}''$, where R' and R'' are as defined hereinabove.

25 Thus, preferred compounds which conform to the above illustratively described general formula, and which can be used for implementing the present invention, are therefore indole derivatives, being compounds having an aromatic ring fused to a heterocyclic ring having at least one nitrogen atom. The parent compound, indole, is a heteroaromatic compound having a phenyl ring fused to a pyrrole ring and
30 thus comprises a completely conjugated pi-electron system.

However, an indole derivative, according to the present invention, encompasses any aromatic moiety that is fused to a heterocyclic ring containing one or more nitrogen atoms (for example, one, two or three nitrogen atoms). Depending of

the location of the pi-electrons of the double bond (between X and Y or Y and Z, see, the formula above) and the nature of the ring atoms (carbon and/or nitrogen), the electronic structure of an indole derivative according to the present invention can include either a partially or completely conjugated pi-electron system.

5 Thus, an indole derivative, according to the present invention, encompasses, for example, substituted or unsubstituted indoles, substituted or unsubstituted purines, substituted or unsubstituted carbazoles and substituted or unsubstituted phenyl ring fused to a substituted or unsubstituted imidazole, pyrazole, thiazine, and the like, with substituted or unsubstituted indoles being the presently preferred indole derivatives.

10 Thus, preferred compounds which can be used for implementing the present invention, are compounds which have the above illustratively described general formula, wherein each of X and Y is carbon, and Z is nitrogen, whereby the double bond (dashed line) is preferably between X and Y.

 Further preferred compounds for implementing the present invention, are
15 compounds which have the above illustratively described general formula, wherein one or more of R_1 - R_{10} comprises an electronegative group. In such compounds, the one or more electronegative groups are directly or indirectly attached to the indole derivative skeleton.

 As used herein, the phrase "electronegative group" describes a chemical moiety
20 or atom which, when bound to another chemical moiety or atom, has at least a partial electronegative charge.

 As can be seen in the Examples section that follows, indole derivatives that include an electronegative group such as hydroxy were found to exhibit superior inhibitory activity and therapeutic effect, as compared with other tested non-hydroxy
25 containing indole derivatives. Without being bound to any particular theory, it is assumed that at least some of the beneficial effects of such compounds can be attributed to the modulation of the electrical properties of the indole derivatives, induced by such an electronegative group.

 Representative examples of electronegative groups that can be beneficially
30 included in the indole derivatives described herein include, without limitation, chemical moieties or atoms that include a halogen atom, an oxygen atom, a sulfur atom, a nitrogen atom, a phosphorus atom and the like, as well as chemical moieties that include a carbon atom adjacent to an electropositive group such as a metal. These

include, for example, a halo group, a hydroxy group, a thiohydroxy group, an amine group, a nitro group, a nitrile group, a sulfonate group, a phosphonate group and the like.

Preferred electronegative groups according to the present embodiments include
5 halo and hydroxy, as these terms are defined herein.

Thus, particularly preferred compounds which can be used for implementing the present invention, are indoles substituted by an electronegative group such as a hydroxy and/or halo group and are therefore compounds which have the above illustratively described general formula, wherein each of X and Y is carbon, and Z is
10 nitrogen, the double bond (dashed line) is between X and Y, and at least one of R_1 - R_{10} is a hydroxy and/or halo group. Preferably, in such hydroxy and/or halo group containing compounds, at least one of R_1 , R_3 , R_4 , and R_9 is a hydroxy and/or halo group, and more preferably, R_1 and/or R_9 is a hydroxy and/or halo group. More preferably, in such hydroxy group containing compounds, each of R_2 - R_5 and R_7 is
15 hydrogen and R_6 , R_8 and R_{10} are absent.

A representative example of such a halo containing compound is 3-haloindole, such that in the general formula, R_1 is hydrogen and R_9 is halo, wherein the halo can be chloro, bromo and iodo. Another representative example of such a halo containing compound is 4-haloindole, such that in the general formula, R_1 is halo and R_9 is
20 hydrogen, wherein the halo can be chloro, bromo and iodo.

Additional particularly preferred compounds which can be used for implementing the present invention, are indoles substituted by one or more halo-containing groups and are therefore compounds which have the above illustratively described general formula, wherein each of X and Y is carbon, and Z is nitrogen, the
25 double bond (dashed line) is between X and Y, and at least one of R_1 - R_{10} is a halo-containing group, as detailed hereinabove. The halo-containing group can be, for example, alkyl, alkenyl, cycloalkyl or aryl being substituted by one or more halo groups.

Preferably, in such halo containing compounds, at least one of R_7 and R_9 is a
30 halo containing group, as detailed hereinabove. More preferably, in such halo containing compounds, each of R_1 - R_5 is hydrogen, and R_6 , R_8 and R_{10} are absent.

As mentioned hereinabove and is further demonstrated and discussed in the Examples section that follows, indole derivatives that include a hydroxy group where found to exhibit a superior inhibitory activity and therapeutic effect.

Thus, preferred compounds for implementing the present invention, are compounds which have the above illustratively described general formula, wherein one or more of R_1-R_{10} comprises a hydroxy group. In such compounds, the one or more hydroxy groups are directly or indirectly attached to the indole derivative skeleton, such that at least one of R_1-R_{10} is either hydroxy or, for example, a hydroxyalkyl, as defined hereinabove.

Particularly preferred compounds which can be used for implementing the present invention, are indoles substituted by a hydroxy group and are therefore compounds which have the above illustratively described general formula, wherein each of X and Y is carbon, and Z is nitrogen, the double bond (dashed line) is between X and Y, and at least one of R_1-R_{10} is a hydroxy group. Preferably, in such hydroxy group containing compounds, at least one of R_1 , R_3 , R_4 , and R_9 is a hydroxy group, and more preferably, R_1 or R_9 is a hydroxy group. More preferably, in such hydroxy group containing compounds, each of R_2-R_5 and R_7 is hydrogen and R_6 , R_8 and R_{10} are absent.

A representative example of such a hydroxy containing compound is 3-hydroxyindole, such that in the general formula, R_1 is hydrogen and R_9 is the hydroxy group. Another representative example of such a hydroxy containing compound is 4-hydroxyindole, such that in the general formula, R_1 is the hydroxy group and R_9 is hydrogen.

Additional particularly preferred compounds which can be used for implementing the present invention, are indoles substituted by one or more hydroxyalkyl groups and are therefore compounds which have the above illustratively described general formula, wherein each of X and Y is carbon, and Z is nitrogen, the double bond (dashed line) is between X and Y, and at least one of R_1-R_{10} is a hydroxyalkyl. Preferably, in such hydroxyalkyl containing compounds, at least one of R_7 and R_9 is a hydroxyalkyl. More preferably, in such hydroxyalkyl containing compounds, each of R_1-R_5 is hydrogen, and R_6 , R_8 and R_{10} are absent. More preferably, in such hydroxyalkyl containing compounds, at least one of R_7 and R_9 is a hydroxymethyl type of hydroxyalkyl.

A representative example of such a hydroxyalkyl containing compound is indole-3-carbinol (3-hydroxymethyl indole), such that in the general formula, R₇ is hydrogen and R₉ is a hydroxymethyl.

Another representative example of such a hydroxyalkyl containing compound
5 is a 2,3-dihydroxyalkyl indole, such that in the general formula above, each R₇ and R₉ is a hydroxyalkyl.

As described in detail in the Examples below, while reducing the present invention to practice, the present inventors empirically determined that amyloid formation can be strongly inhibited by each of the indole derivatives indole-3-carbinol,
10 3-hydroxyindole, or 4-hydroxyindole, individually. This empirical determination confirms the inventive concept that compounds having an indole moiety, as in tryptophane, would participate in the aromatic interactions described above and would thus inhibit amyloid formation, thereby suggesting the use of these compounds in the treatment of amyloid associated diseases.

Without being bound to any particular theory, it is assumed that the hydroxy
15 group, by being an electron-donor group, alters the electron density and the negative charge on the benzopyrrole ring. Additionally, the hydroxy group is capable of interacting with various moieties in the polypeptide by a means of hydrogen bonds. The position of the hydroxyl group is also of key importance. Thus, while the
20 amyloid formation inhibitory mechanism of the indole derivatives described herein remains unclear, it is assumed that the hydroxyl group interacts with the backbone of the peptides, and prevents the occurrence of π -stacking interactions between the peptides by providing a different electron density and a negative charge of the benzopyrrole and thus the extension of the fibrillogenesis process is blocked.

Thus, according to a main aspect of the present invention, there is provision of
25 a method of treating an amyloid associated disease in a subject. The method includes administering to a subject in need thereof, a therapeutically effective amount of the compound described hereinabove, a pharmaceutically acceptable salt thereof, or a prodrug thereof, thereby treating the amyloid associated disease in the subject.

30 Preferred individual subjects according to the present invention are mammals such as canines, felines, ovines, porcines, equines, and bovines. Preferably the individual subjects according to the present invention are humans.

The term 'treating' refers to reducing or preventing amyloid plaque formation, or substantially decreasing plaque occurrence in an affected tissue. The phrase 'amyloid plaque' refers to fibrillar amyloid as well as aggregated but not fibrillar amyloid, hereinafter 'protofibrillar amyloid', which may be pathogenic as well [see
5 Anaguiano et al. (2002) Biochemistry 41:1 1338-43].

Amyloid associated diseases treated according to the present invention include, but are not limited to, type II diabetes mellitus, Alzheimer's disease (AD), early onset Alzheimer's disease, late onset Alzheimer's disease, presymptomatic Alzheimer's disease, Parkinson's disease, SAA amyloidosis, hereditary Icelandic
10 syndrome, multiple myeloma, medullary carcinoma, aortic medical carcinoma, Insulin injection amyloidosis, prion-systematic amyloidosis, choronic inflammation amyloidosis, Huntington's disease, senile systemic amyloidosis, pituitary gland amyloidosis, Hereditary renal amyloidosis, familial British dementia, Finnish hereditary amyloidosis, familial non-neuropathic amyloidosis [Gazit (2002) Curr.
15 Med. Chem. 9:1667-1675] and prion diseases including scrapie of sheep and goats and bovine spongiform encephalopathy (BSE) of cattle [Wilesmith and Wells (1991) Curr Top Microbiol Immunol 172: 21-38] and human prion diseases including (i) kuru, (ii) Creutzfeldt-Jakob Disease (CJD), (iii) Gerstmann-Streussler-Sheinker Disease (GSS), and (iv) fatal familial insomnia (FFI) [Gajdusek (1977) Science 197:
20 943-960; Medori, Tritschler et al. (1992) N Engl J Med 326: 444-449].

The compounds described hereinabove can be administered or otherwise utilized in this and other aspects of the present invention, either as is, or as a pharmaceutically acceptable salt, or a prodrug thereof.

The term 'prodrug' refers to an agent, which is converted into the active
25 compound (the active parent drug) *in vivo*. Prodrugs are typically useful for facilitating the administration of the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent drug is not. The prodrug may also have improved solubility as compared with the parent drug in pharmaceutical compositions. Prodrugs are also often used to achieve a sustained release of the
30 active compound *in vivo*. An example, without limitation, of a prodrug would be a compound of the present invention, having one or more phenol moieties, which is administered as an ester (the 'prodrug'). Such a prodrug is hydrolysed *in vivo*, to

thereby provide the free compound (the parent drug). The selected ester may affect both the solubility characteristics and the hydrolysis rate of the prodrug.

The phrase 'pharmaceutically acceptable salt' refers to a charged species of the parent compound and its counter ion, which is typically used to modify the solubility characteristics of the parent compound and/or to reduce any significant irritation to an organism by the parent compound, while not abrogating the biological activity and properties of the administered compound. An example, without limitation, of a pharmaceutically acceptable salt would be an alkoxide anion (RO^- , where R is alkyl or cycloalkyl as defined hereinabove) and a cation such as, but not limited to, ammonium, sodium, potassium and the like. Preferably, the compound of the present invention is administered at a concentration not exceeding 4 mg/kg x hr.

It will be appreciated that treatment of amyloid associated diseases according to the present invention may be combined with other treatment methods known in the art (i.e., combination therapy). Thus, compounds of the present invention may be co-administered (simultaneously or separately) with additional anti-amyloid drugs. Examples of such anti-amyloid drugs include, but are not limited to, amyloid destabilizing antibodies, amyloid destabilizing peptides and anti-amyloid small molecules (further details on such drugs are provided in the preceding Background section).

According to another aspect of the present invention, there is provided use of the indole derivatives described above for the manufacture of a medicament identified for the treatment of amyloid associated diseases.

According to still another aspect of the present invention there is provided a pharmaceutical composition including a therapeutically effective amount of the compound described hereinabove, and a pharmaceutically acceptable carrier.

Accordingly, the compounds of the present invention can be provided to an individual *per se*, or as part of a pharmaceutical composition where it is mixed with a pharmaceutically acceptable carrier.

As used herein a 'pharmaceutical composition' refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to the subject treated.

Herein the term 'active ingredient' refers to the compound, which is accountable for the biological effect.

Hereinafter, the phrases 'physiologically acceptable carrier' and 'pharmaceutically acceptable carrier' which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to the subject and does not abrogate the biological activity and properties of the administered compound. Preferred carriers of the pharmaceutical composition of the present invention include, but are not limited to, polyethylene glycol (PEG), a biocompatible polymer with a wide range of solubility in both organic and aqueous media (Mutter et al. (1979).

Herein the term 'excipient' refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in 'Remington's Pharmaceutical Sciences,' Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

Alternately, one may administer a preparation in a local rather than systemic manner, for example, via injection of the preparation directly into a specific region of a patient's body.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing

of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal
5 administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well
10 known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable
15 auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers
20 such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic,
25 talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions, which can be used orally, include push-fit
30 capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft

capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. AU formulations for oral administration should be in dosages suitable for the chosen route of administration.

5 For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by nasal inhalation, the active ingredients for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable
10 propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

15 The preparations described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain
20 formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame
25 oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly
30 concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

The preparation of the present invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

Pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of active ingredients effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from *in vitro* assays. For example, a dose can be formulated in animal models and such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures *in vitro*, in cell cultures or experimental animals. The data obtained from these *in vitro* and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. [See e.g., Fingl, et al., (1975) "The Pharmacological Basis of Therapeutics", Ch. 1 p.1].

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions including the preparation of the present invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

According to another aspect of the present invention, there is provided an article-of-manufacture including a packaging material and a pharmaceutical composition identified for treating amyloid associated diseases being contained within the packaging material, the pharmaceutical composition including, as an active ingredient, the compound described hereinabove, and a pharmaceutically acceptable carrier.

Compositions of the present invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert.

It is to be fully understood that the present invention further encompasses all of the compounds described hereinabove, in accordance with the above illustratively described general formula.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non-limiting fashion.

MATERIALS AND EXPERIMENTAL METHODS

Peptides and Chemicals:

5 Recombinant β -amyloid ($A\beta$ 1-40, > 98% pure) polypeptide was purchased from rPeptide (Athens GA, USA). Indole-3-carbinol (Figure 1) was purchased from Sigma. All reagents were of analytical grade.

Thioflavin T fluorescence assay:

10 Fibrillization of $A\beta$ 1-40 polypeptide was monitored using a Thioflavin T (ThT) dye binding assay. $A\beta$ 1-40 stock solution was diluted to a final concentration of 5 μ M polypeptide in 100 nM NaCl, 10 mM sodium phosphate buffer (pH 7.4), without and with addition of the indole-3-carbinol inhibitor (10 μ M). For each measurement, ThT was added to a 0.1 ml sample, to give a final concentration of 0.3 μ M ThT and 0.4 μ M $A\beta$ 1-40 polypeptide. Fluorescence measurements, made after
15 addition of the ThT solution to each sample at 37 °C, were carried out using a Jobin Yvon FluroMax-3 spectrometer (excitation 450 nm, 2.5 nm slit; emission 480 nm, 5 nm slit, integration time of 1 second). Background was subtracted from each sample.

20 *Transmission Electron Microscopy (TEM) Measurements:* 10 μ l samples from the ThT fluorescence assay were placed on 400-mesh copper grids (SPI supplies, West Chester PA) covered by carbon stabilized Formvar film. After 1 minute, excess fluid was removed, and the grids were negatively stained with 2 % uranyl acetate in water for another two minutes. Microstructural features of the samples were viewed using a JEOL 1200EX electron microscope operating at 80 kV.

25 EXPERIMENTAL RESULTS

 The results obtained in this example clearly show that indole-3-carbinol, as an exemplary indole derivative according to the present invention, effectively inhibits formation of β -amyloid polypeptide fibrils.

30 The level of amyloid formation was detected using the above described Thioflavin T (ThT) dye binding assay. Fluorescence (intensity, measured in units of cps (counts per second)) of the ThT bound $A\beta$ 1-40 polypeptide, in the absence and in the presence of 10 μ M indole-3-carbinol, was monitored over time, for up to about

350 hours. The results, representing a mean of two independent measurements, are graphically presented in Figure 2.

As is shown in Figure 2, A β 1-40 polypeptide without the indole-3-carbinol (squares) displayed a typical lag-phase of about 100 hours, followed by a rapid increase in fluorescence that reached a plateau after about 200 hours. In the presence of 10 μ M indole-3-carbinol (triangles), following about 100 hours, significantly lower levels of fluorescence were detected for the remaining duration of the assay. The lag-time appeared to be significantly extended and the attainment of a plateau was only after about 300 hours. Furthermore, the level of fluorescence at the plateau in the presence of indole-3-carbinol was about a third of the value as compared to that of the non-inhibited A β 1-40 polypeptide.

In order to obtain insight into the mechanism of inhibiting formation of β -amyloid polypeptide type of amyloid fibrils, representative samples taken from the ThT fluorescence assay measurements described above after attainment of the plateau (at about 192 hours, Figure 2), in the absence or presence of indole-3-carbinol, were viewed using electron microscopy.

The obtained micrographs, presented in Figures 3a and 3b demonstrated that distinct and well defined amyloid fibers were present in samples of A β 1-40 polypeptide in the absence of indole-3-carbinol (Figure 3a), while, by strong contrast, when indole-3-carbinol was present as an inhibitor in the A β 1-40 polypeptide sample, significantly shorter and less dense fiber type material was observed (Figure 3b). The overall amount of aggregated fibrous material on the electron microscope grid appeared to be significantly lower in the presence of the indole-3-carbinol compound.

25

EXAMPLE 2

MATERIALS AND EXPERIMENTAL METHODS

A β peptides:

A β solutions: Synthetic lyophilized β -amyloid polypeptides (A β 1-40 and A β 1-42, > 98% pure) were purchased from Bachem (Bubendorf, Switzerland). A 100 μ M A β stock solution was prepared by dissolving A β 1-40 or A β 1-42 in

30

dimethylsulfoxide (DMSO) and sonicating the resulting solution during 1 minute to avoid pre-aggregation. A β solutions were prepared by immediate dilution with 10 mM phosphate-buffered saline (100 mM NaCl, 0.5 mM EDTA, pH 7.4) to a final concentration of 10 μ M (containing 10 % (v/v) DMSO).

5 **Indole derivatives:** The following indole derivatives were obtained from Sigma-Aldrich Inc.: Indole-3-carbynol, Tryptamine, 5-nitroindole, 7-methylindole, 5-methylindole, 3-methylindole, indole-3-butyric acid, indole-3-acetic acid, indole-6-carboxylic acid, indole-5-carboxylic acid, indole-3-carboxylic acid, indole-2-carboxylic acid, 2-indolinone, 3-hydroxyindole, 4-hydroxyindole, 5-hydroxyindole,
10 Fmoc-D-Trp, Boc-L-T ϕ -OH, CBZ-D-Tryptophanamide, CBZ-L-Tryptophanamide, L-5-hydroxytryptophan, D-Tryptophan, L-Tryptophan, indole-3-propionic acid indole-3-ethanol, indole, N α -Boc-D-Trp, N α -Boc-L-T ϕ , Fmoc-Trp-OH.

Indole derivatives stock solutions were prepared by dissolving an indole derivative in DMSO, so as to achieve a concentration of 20 mM and then diluting the
15 solution with 10 mM PBS buffer (100 mM NaCl, 0.5 mM EDTA, pH 7.4) to a final concentration of 100 μ M (containing 0.5 % (v/v) DMSO).

Thioflavin T fluorescence assay: Fibrillization of A 1-42 polypeptide was monitored using a Thioflavin T (ThT) dye binding assay. A 10 μ M A β solution (either A β 1-40 or A β 1-42) was prepared as described above and was immediately
20 mixed with the indole derivative stock solutions (100 μ M), so as to achieve a final concentration of 5 μ M for A β and various concentrations of the indole derivative. For each measurement, ThT was added to a 0.1 ml sample, to give a final concentration of 0.3 μ M ThT and 0.4 μ M A . The samples were incubated at 37° C. Fluorescence measurements, made after addition of the ThT solution to each sample at 37 °C, were
25 carried out using a Jobin Yvon FluroMax-3 spectrometer (excitation 450 nm, 2.5 nm slit; emission 480 nm, 5 nm slit, integration time of 1 second). Background was subtracted from each sample. Each experiment was repeated in quadruplicate.

Transmission Electron microscopy (TEM): A 10 μ l sample of the ThT fluorescence assay was placed on a 400 mesh copper grid covered by carbon-stabilized Formvar film (SPI Supplies, West Chester, PA). After 1.5 minutes, excess
30 fluid was removed and the grids were negatively stained for 2 minutes with 10 μ l of 2 % uranyl acetate solution. Excess fluid was removed and the samples were viewed using a JEOL 1200EX electron microscope operating at 80 kV.

Atomic force microscopy (AFM): For each experiment, a 60 μ l sample of was taken for AFM measurement at three different time points correlating to the amyloidogenic kinetic curve, as detailed hereinafter. The sample was centrifuged for 1.5 minutes at 13,000 rpm and 40 μ l of the supernatant was removed. The remaining
5 20 μ l droplet of the solution was re-suspended and deposited onto freshly cleaved mica for 1.5 minutes. The substrate was washed with 160 μ l of double-distilled H₂O to reduce background and to eliminate salts and buffer contaminants. The mica samples were then dried at room temperature. At least four regions of the mica surface were examined to ensure that similar structures existed throughout the sample.
10 AFM images were obtained using a Veeco NanoScope IV MultiMode AFM (Digital Instruments, Santa Barbara, USA) in tapping mode. Measurements were performed at a resonance frequency of about 321.4 kHz.

EXPERIMENTAL RESULTS

15 The results obtained in this example clearly show that various exemplary indole derivatives, according to the present embodiments, effectively inhibit formation of β -amyloid polypeptide fibrils.

Inhibition of amyloid formation: The level of amyloid formation was detected using the above described Thioflavin T (ThT) dye binding assay.
20 Fluorescence (relative intensity, measured in units of percent) of the ThT bound A β 1-42 remaining following 24 hours at 37 °C, in the absence, and in the presence of 50 μ M indole or indole derivative, was measured. Each measurement was recorded immediately after the fluorescence intensity reached a maximum value. The results obtained with representative indole derivatives, each representing a mean of four
25 independent measurements along with an associated error bar representing a standard error, are graphically presented in Figure 4.

As is shown in Figure 4, indole-3-carbinol (denoted as IC), 3-hydroxyindole (denoted as 3HI), and 4-hydroxyindole (denoted as 4HI), individually, significantly inhibited β -amyloid polypeptide fibril formation.

30 These results indicate that an effective inhibition of amyloid formation is particularly observed with indole derivatives that include one or more hydroxy groups. In each case of effective inhibition of amyloid formation, the indole derivative has a hydroxy (-OH) group, either directly (3-hydroxyindole, 4-

hydroxyindole), or via a carbon linker (indole-3-carbinol), attached to the indole ring. Indole derivatives having, for example, a carboxylic acid or an alkyl attached directly or indirectly to the indole ring, did not exhibit an effective inhibition of amyloid formation.

5 Moreover, it appears that the inhibition functionality of the indole derivatives is further affected by the exact position of the -OH group, since, for example, 2-hydroxyindole (denoted as 2HI) or 5-hydroxyindole (denoted as 5HI) showed a significantly reduced inhibition of amyloid formation, as compared with indole-3-carbinol (denoted as IC), 3-hydroxyindole (denoted as 3HI), and 4-hydroxyindole
10 (denoted as 4HI).

 These results therefore further support the concept according to which the inhibition of amyloid formation is effected by aromatic interactions (induced by the indole skeleton) and is presumably further affected by hydrogen bonds, induced by the hydroxy group(s). These results further indicate that the distance between the
15 aromatic moiety and the hydroxy group affects the inhibition activity, as demonstrated by the differential activity between 3HI, IC, and IE.

Concentration Dependence of Indole Derivatives inhibition activity: To examine the inhibition efficiency of the indole derivatives, the concentration dependence of inhibition of fibril formation was studied. The fibrillization of A β 1-42,
20 with and without an indole derivative was measured using the ThT fluorescence assay described above at a fixed incubation time of 24 hours. The results are presented in Figure 5.

 As shown in Figure 5, the 3-hydroxyindole (denoted as 3HI), and 4-hydroxyindole (denoted as 4HI) clearly showed a dose-dependent inhibition pattern.
25 The indole-3-carbinol (denoted herein as IC) inhibited the fibrillization of A β 1-42 only at high concentrations and showed no dose-dependency.

 The IC₅₀ values of each compound was calculated by the curve equation. The following apparent IC₅₀ values were obtained: about 85 μ M for 4-hydroxyindole; about 100 μ M for 3-hydroxyindole, and about 200 μ M for indole-3-carbinol.

30 In order to rule out the possibility that the low values obtained in the ThT fluorescence assay may result from a quenching effect by the indole derivative a control experiment was conducted as follows: different concentrations of the three indole derivatives were added to form A β 1-42 fibrils and fluorescence was

immediately measured. No significant quenching effect was observed (data not shown).

Kinetics Studies of A β 1-40 Fibrillogenesis Inhibition: A β 1-40, which has a slower fibrillogenesis rate [see, Jarrett et al. (1993) *Biochemistry* 32, 4693-4697] was used in these studies, in order to obtain better temporal resolution, assuming that a slower rate would allow more significant inhibition and a better understanding of the effect. 3-Hydroxyindole (denoted as 3HI), 4-hydroxyindole (denoted as 4HI) and indole-3-carbinol (denoted herein as I3C) were each added at concentrations of 5 μ M, 25 μ M, and 50 μ M to a solution containing 5 μ M of A β 1-40 (prepared as described above) and the kinetics of A β 1-40 fibrillogenesis inhibition was measured using the ThT fluorescence assay.

The results obtained with 4-hydroxyindole are presented in Figure 6a. The results obtained with 3-hydroxyindole are presented in Figure 6b. The results obtained with indole-3-carbinol are presented in Figure 6c. As is shown in Figures 6a-6c the ThT fluorescence curve obtained for all the tested indole derivatives showed a characteristic sigmoid shape representing three phases: the nucleation phase (lag phase), the polymerization phase, and the equilibrium phase (stationary phase).

As shown in Figure 6a, samples containing 25 μ M and 50 μ M 4-hydroxyindole showed a significantly longer nucleation phase, which persisted throughout the duration of the measurements. In the sample containing 5 μ M of 4-hydroxyindole, the effect on the nucleation phase was less significant.

As shown in Figures 6b and 6c, the samples containing 3-hydroxyindole and indole-3-carbinol, respectively, had no effect on the nucleation phase when compared with the control.

As is further shown in Figures 6a-6c, all the tested indole derivatives were inhibitory in the polymerization phase, such that with all the tested compounds the slope of the curve was changed, although with different magnitudes. Thus, I3C had an effect on the slope of the growth curve only at 50 μ M whereby no effect was observed at lower concentrations (Figure 6c). 3HI showed a dose-dependent effect, in which the slope of the growth curve changed in parallel with increasing concentrations of 3HI (Figure 6b). The slope of the samples containing 5 μ M 4HI was the most sharp, as compared with that of the control or the other tested indole derivatives.

Differences in the level of ThT fluorescence at the equilibrium phase are influenced by the ability of the indole derivative to redirect the A β away from the polymerization pathway. As shown in Figures 6a-6c, at the final equilibrium phase (at the endpoint of the experiments), 4HI exhibited the highest inhibition level at all the tested concentrations. The inhibition level of 4HI at 5 μ M was almost 60 %, whereby at 25 μ M and 50 μ M the inhibition level was almost complete (Figure 6a). The inhibition level of 3HI at 25 μ M was about 40 %, and was about 60 % at 50 μ M (Figure 6b). The inhibition level of DC at 50 μ M B C was about 50 % (Figure 6c).

While recent evidence has indicated that the toxicity of A β and other amyloidogenic proteins lie in the soluble oligomeric forms [see, for example, Lambert et al. (1998) *Proc. Natl. Acad. Sci. U.S.A.* 95, 6448-6453; Wang et al. (2002) *Brain Res.* 924, 133-140; Walsh et al. (2002) *Biochem. Soc. Trans.* 30, 552-557; Kim, et al. (2003) *FASEB J.* 17, 118-120; Kaye et al. (2003) *Science* 300, 486-489; and Cleary et al. (2004) *Nature Neurosci.* 8, 79-84], notion has been made to the potential effect of anti-amyloid compounds in blocking A β oligomerization at a very early stage, most likely the neurotoxic stage.

The kinetic studies described herein are essential for determining the phases inhibited by each indole derivative and indeed have shown the ThT fluorescence assay is consistent with the nucleation-dependent polymerization model [Jarrett and Lansbury, (1993) *Cell* 73, 1055-1058; and Naik et al. (1997) *Biochemistry* 36, 6243-6250]. The results obtained in these studies have showed that the indole derivative changes the shape of the fibrillogenesis curve in three ways: (i) interference with nucleation, leading to increase of the lag time; (ii) interference during the polymerization step, which significantly slows down the polymerization rate; and (iii) inhibition in which A β is diverted from the polymerization pathway, reducing the endpoint equilibrium level.

The results in these assays show that I3C and 3HI interfere in the polymerization stage and divert the process away from that pathway. 4HI seemed to interfere in all three steps.

Ultrastructural Analysis of amyloid fibril formation: The effect of the indole derivatives on the ultrastructural properties of the assembled A β was first evaluated by TEM measurements. Thus, TEM analysis was performed with samples

of A β 1-40 obtained from the kinetic studies described above after incubation for 14 days.

The obtained micrographs of A β 1-40 are presented in Figure 7a (control), Figure 7b (following 14 days incubation in the presence of 25 μ M 4-hydroxyindole), Figure 7c (following 14 days incubation in the presence of 50 μ M) and Figure 7d (following 14 days incubation in the presence of 50 μ M indole-3-carbonil). As shown in Figure 7a, control samples of A β 1-40 contained abundant amyloid fibrils aggregated in PBS. As shown in Figure 7c, samples containing A β 1-40 and 25 μ M 4HI were devoid of fibrils and contained only a minor amount of small fibrils. As shown in Figure 7c, samples containing A β 1-40 and 50 μ M 3HI had a significant number of amorphous aggregates showing a congested form. It is assumed that the latter may affect the ThT measurements as a result of binding. As shown in Figure 7d, samples containing A β 1-40 and 50 μ M I3C resulted in the same form as that observed in the control sample, although smaller amounts of amyloid fibril aggregates were observed.

These results stand in correlation with the above described quantitative fluorescence assays, and indicate that the only grids showing no amyloid fibrils were those containing samples of A β and 5 μ M or more of 4HI.

Additional structural analyses were performed using AFM measurements, performed with A β 1-40 samples taken from the kinetic studies once the control samples reached the different fibrillogenesis phase. None of the analyzed samples immediately showed amyloid fibrils (data not shown).

AFM measurements performed with A β 1-40 samples taken from the kinetic studies once the control samples reached the polymerization phase showed different levels of amyloid fibril formation, as shown in Figures 8a-d. Thus, small amounts of amyloid fibrils in the form of long wires were observed in the control sample (Figure 8a) and in the sample containing I3C (Figure 8d), whereas samples containing A β 1-40 and 4HI showed no fibrils (Figure 8b). In samples containing 3HI a different fibril morphology was observed. As shown in Figure 8c, in these samples small aggregates that interacted with the amyloid fibrils were observed.

These results therefore indicate that all the tested AFM samples were clear of fibrils in the immediate lag phase, and only those containing 4HI remained clear of amyloid fibrils in the following polymerization phase. These results support those of

the kinetic assay in which the 4HI lag time was longer than in other samples and may suggest that 4HI blocks A β fibrillogenesis in the early stages of nucleation.

An apparent paradox was shown in the results obtained with 3-hydroxyindole. While a significant decrease occurred in the A β fibrillogenesis kinetic assay, this compound had no anti-cytotoxic effect when tested with cultured PC12 cells, as discussed in detail hereinbelow. Without being bound to any particular theory, it is assumed that the variant morphology among the amorphous aggregates shown in the TEM micrograph (Figure 7c) may be the reason for such a discrepancy.

EXAMPLE 3

MATERIALS AND EXPERIMENTAL METHODS

Cell culture: PC12 pheochromocytoma cell line, a cultured benign tumor of the sympathetic nervous system, was routinely grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 8 % (v/v) fetal calf serum, 8 % (v/v) horse serum, 100 U/ml penicillin, 100 U/ml streptomycin, and 2 mM L-glutamine (Biological industries, Israel). The cells were maintained at 37 °C in a humidified atmosphere containing 5 % CO₂.

Cell cytotoxicity assay: PC-12 cells (3 x 10⁵ cells/ml) were cultured in 96-well microplates (100 μ l/well) and were incubated overnight at 37 °C. The wells were then washed once with serum free-DMEM, so as to exclude the effect of the serum. To each well 100 μ l of 5 μ M A β 1-40 (prepared from a stock solution of 0.5 mM in 10 mM NaOH) and an indole derivative at various concentrations were added. Each determination was made in quadruplicate. Following incubation for 24 hours at 37 °C, cell viability was evaluated using the MTT assay (as described in Hansen et al. (1989) *J. Immunol. Methods* 119, 203-210).

EXPERIMENTAL RESULTS

The effect of the indole derivatives on the formation of cytotoxic amyloid assemblies was determined by measuring the toxicity of the formed A β on pheochromocytoma cell line cultures (PC12) in the presence and absence of the indole derivatives. Thus, solutions of 5 μ M A β 1-40 and various concentrations of the indole

derivatives 3HI, 4HI and I3C were prepared and added to the cells at two time points: immediately ($T = 0$) or 7 days after incubation. Cell viability was determined after 24 hours using the MTT assay. Cells incubated with only 5 μM A β 1-40 served as the control.

5 The results obtained following addition of the above solutions immediately after their preparation ($T = 0$) represent the cytotoxic effect during the first 24 hours of A β fibrillogenesis and are presented in Figure 9a. The results obtained following addition of the above solutions after 7 days incubation ($T = 7$) represent the cytotoxic effect at the end point phase, when large and congested amyloid fibrils were already
10 formed, and are presented in Figure 9b.

As shown in Figure 9a, the average survival rate at $T=0$ with various concentrations of the various indole derivatives, was about 56 %. As shown in Figure 9b, at $T=7$, the survival rate of the control was about 74 %. This survival rate was higher than that at $T=0$.

15 As is further shown in Figures 9a-b, 4HI had the most significant inhibitory effect on A β 1-40 cytotoxicity in both cell culture assays. Thus, for example, upon addition of solutions containing A β 1-40 and 25 or 50 μM of 4HI, the viability rate of PC12 cells was elevated by about 25 % as compared with the control. Addition of solutions containing 3HI, improved the PC12 survival rate by about 25 % as
20 compared with the control only at $T=0$. No significant inhibition of cytotoxicity was observed with I3C in either assay.

When solutions containing only the indole derivatives were added to the cells, no effect on the survival of PC 12 cells was observed (data not shown).

The protective effect of 4HI was further defined by adding solutions
25 containing 5 μM A β 1-40 and various concentrations of 4HI, ranging from 0.5 μM to 50 μM , to PC-12 cells, following 7 days incubation of the solution. The results are presented In Figure 10 and clearly show that the protective effect of 4HI is dose-dependent. The obtained results further indicate that the minimum concentration of 4HI required for significant inhibition was 2.5 μM , whereas the toxic effect of A β 1-40
30 was completely blocked at 50 μM 4HI.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention,

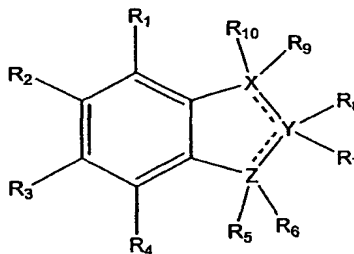
which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

WHAT IS CLAIMED IS:

1. A method of treating an amyloid associated disease in a subject, the method comprising administering to a subject in need thereof, a therapeutically effective amount of a compound having the general formula:



a pharmaceutically acceptable salt thereof, or a prodrug thereof,
wherein:

the dashed line denotes a double bond either between X and Y, or, between Y and Z;

X, Y and Z are each independently selected from the group consisting of carbon and nitrogen, whereas at least one of X, Y, and Z is nitrogen; and

R_1 - R_{10} are each independently selected from the group consisting of hydrogen, lone pair electrons, hydroxy, alkyl, cycloalkyl, phenyl, phenol, hydroxyphenol, dihydroxyphenol, aryl, alkenyl, alkynyl, heteroaryl, heteroalicyclic, halo, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, C-carboxy, O-carboxy, thiocarboxy, carbonyl, oxo, thiocarbonyl, sulfinyl, and sulfonyl, or absent, or, alternatively, at least two of R_1 - R_{10} form at least one five- or six-membered aromatic, heteroaromatic, alicyclic or heteroalicyclic ring,

thereby treating the amyloid associated disease in the subject.

2. The method of claim 1, wherein:

each of X and Y is carbon; and

Z is nitrogen.

3. The method of claim 2, wherein said double bond is between X and Y.

4. The method of claim 1, wherein at least one of R_1 - R_{10} comprises an electronegative group.
5. The method of claim 1 or 3, wherein at least one of R_1 and R_9 comprises an electronegative group.
6. The method of any of claims 4 and 5, wherein said electronegative group is selected from the group consisting of hydroxy and halo.
7. The method of claim 1, wherein at least one of R_1 - R_{10} comprises a hydroxy group.
8. The method of claim 3, wherein at least one of R_1 - R_9 comprises a hydroxy group.
9. The method of claim 8, wherein at least one of R_1 and R_9 is a hydroxy group.
10. The method of claim 9, wherein each of R_2 - R_5 and R_7 is hydrogen and R_6 , R_8 and R_{10} are absent.
11. The method of claim 10, wherein R_1 is hydrogen and R_9 is a hydroxy group.
12. The method of claim 10, wherein R_1 is a hydroxy group and R_9 is hydrogen.
13. The method of claim 8, wherein at least one of R_1 - R_{10} is a hydroxyalkyl.
14. The method of claim 13, wherein at least one of R_7 and R_9 is a hydroxyalkyl.

15. The method of claim 14, wherein each of R_1 - R_5 is hydrogen and R_6 , R_8 and R_{10} are absent.

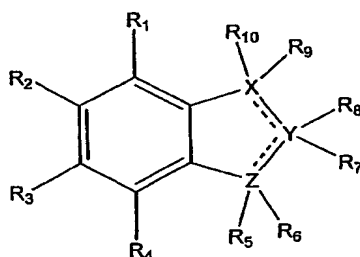
16. The method of claim 14, wherein said hydroxyalkyl is hydroxymethyl.

17. The method of claim 16, wherein R_7 is hydrogen and R_9 is said hydroxymethyl.

18. The method of claim 17, wherein each of R_1 - R_5 is hydrogen and R_6 , R_8 and R_{10} are absent.

19. The method of claim 14, wherein each of R_7 and R_9 is a hydroxyalkyl.

20. Use of a compound having the general formula:



a pharmaceutically acceptable salt thereof, or a prodrug thereof,
wherein:

the dashed line denotes a double bond either between X and Y , or, between Y and Z ;

X , Y and Z are each independently selected from the group consisting of carbon and nitrogen, whereas at least one of X , Y , and Z is nitrogen; and

R_1 - R_{10} are each independently selected from the group consisting of hydrogen, lone pair electrons, hydroxy, alkyl, cycloalkyl, phenyl, phenol, hydroxyphenol, dihydroxyphenol, aryl, alkenyl, alkynyl, heteroaryl, heteroalicyclic, halo, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, C-carboxy, O-carboxy, thiocarboxy,

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carbonyl, oxo, thiocarbonyl, sulfinyl, and sulfonyl, or absent, or, alternatively, at least two of R_1 - R_{10} form at least one five- or six-membered aromatic, heteroaromatic, alicyclic or heteroalicyclic ring,

for the manufacture of a medicament identified for the treatment of amyloid associated diseases.

21. The use of claim 20, wherein:

each of X and Y is carbon; and

Z is nitrogen.

22. The use of claim 21, wherein said double bond is between X and Y.

23. The use of claim 20, wherein at least one of R_1 - R_{10} comprises an electronegative group.

24. The use of claim 20 or 22, wherein at least one of R_1 and R_9 comprises an electronegative group.

25. The use of any of claims 23 and 24, wherein said electronegative group is selected from the group consisting of hydroxy and halo.

26. The use of claim 20, wherein at least one of R_1 - R_{10} comprises a hydroxy group.

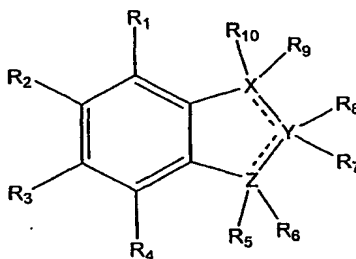
27. The use of claim 22, wherein at least one of R_1 - R_9 comprises a hydroxy group.

28. The use of claim 27, wherein at least one of R_1 and R_9 is a hydroxy group.

29. The use of claim 28, wherein each of R_2 - R_5 and R_7 is hydrogen and R_6 , R_8 and R_{10} are absent.

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30. The use of claim 29, wherein R_1 is hydrogen and R_9 is a hydroxy group.
31. The use of claim 29, wherein R_1 is a hydroxy group and R_9 is hydrogen.
32. The use of claim 27, wherein at least one of R_1 - R_{10} is a hydroxyalkyl.
33. The use of claim 32, wherein at least one of R_7 and R_9 is a hydroxyalkyl.
34. The use of claim 33, wherein each of R_1 - R_5 is hydrogen and R_6 , R_8 and R_{10} are absent.
35. The use of claim 33, wherein said hydroxyalkyl is hydroxymethyl.
36. The use of claim 35, wherein R_7 is hydrogen and R_9 is said hydroxymethyl.
37. The use of claim 36, wherein each of R_1 - R_5 is hydrogen and R_6 , R_8 and R_{10} are absent.
38. The use of claim 33, wherein each of R_7 and R_9 is a hydroxyalkyl.
39. A pharmaceutical composition, for use in the treatment of amyloid associated diseases, comprising a therapeutically effective amount of a compound having the general formula:



a pharmaceutically acceptable salt thereof, or a prodrug thereof,
wherein:

the dashed line denotes a double bond either between X and Y, or, between Y and Z;

X, Y and Z are each independently selected from the group consisting of carbon and nitrogen, whereas at least one of X, Y, and Z is nitrogen; and

R_1 - R_{10} are each independently selected from the group consisting of hydrogen, lone pair electrons, hydroxy, alkyl, cycloalkyl, phenyl, phenol, hydroxyphenol, dihydroxyphenol, aryl, alkenyl, alkynyl, heteroaryl, heteroalicyclic, halo, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, C-carboxy, O-carboxy, thiocarboxy, carbonyl, oxo, thiocarbonyl, sulfinyl, and sulfonyl, or absent, or, alternatively, at least two of R_1 - R_{10} form at least one five- or six-membered aromatic, heteroaromatic, alicyclic or heteroalicyclic ring,

and a pharmaceutically acceptable carrier.

40. The pharmaceutical composition of claim 39, wherein:
each of X and Y is carbon; and
Z is nitrogen.

41. The pharmaceutical composition of claim 40, wherein said double bond is between X and Y.

42. The pharmaceutical composition of claim 39, wherein at least one of R_1 - R_{10} comprises an electronegative group.

43. The pharmaceutical composition of claim 39 or 41, wherein at least one of R_1 and R_9 comprises an electronegative group.

44. The pharmaceutical composition of any of claims 42 and 43, wherein said electronegative group is selected from the group consisting of hydroxy and halo.

45. The pharmaceutical composition of claim 39, wherein at least one of R_1 - R_{10} comprises a hydroxy group.

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46. The pharmaceutical composition of claim 41, wherein at least one of R_1 - R_9 comprises a hydroxy group.

47. The pharmaceutical composition of claim 46, wherein at least one of R_1 and R_9 is a hydroxy group.

48. The pharmaceutical composition of claim 47, wherein each of R_2 - R_5 and R_7 is hydrogen and R_6 , R_8 and R_{10} are absent.

49. The pharmaceutical composition of claim 48, wherein R_1 is hydrogen and R_9 is a hydroxy group.

50. The pharmaceutical composition of claim 48, wherein R_1 is a hydroxy group and R_9 is hydrogen.

51. The pharmaceutical composition of claim 46, wherein at least one of R_1 - R_{10} is a hydroxyalkyl.

52. The pharmaceutical composition of claim 51, wherein at least one of R_7 and R_9 is a hydroxyalkyl.

53. The pharmaceutical composition of claim 52, wherein each of R_1 - R_5 is hydrogen and R_6 , R_8 and R_{10} are absent.

54. The pharmaceutical composition of claim 52, wherein said hydroxyalkyl is hydroxymethyl.

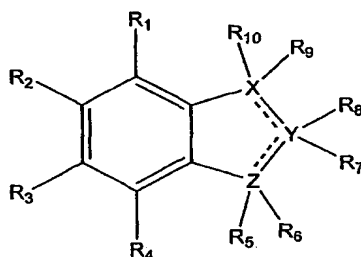
55. The pharmaceutical composition of claim 54, wherein R_7 is hydrogen and R_9 is said hydroxymethyl.

56. The pharmaceutical composition of claim 55, wherein each of R_1 - R_5 is hydrogen and R_6 , R_8 and R_{10} are absent.

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57. The pharmaceutical composition of claim 52, wherein each of R_7 and R_9 is a hydroxyalkyl.

58. An article-of-manufacture comprising a packaging material and a pharmaceutical composition identified for treating amyloid associated diseases being contained within said packaging material, said pharmaceutical composition including, as an active ingredient, a compound having the general formula:



a pharmaceutically acceptable salt thereof, or a prodrug thereof, wherein:

the dashed line denotes a double bond either between X and Y, or, between Y and Z;

X, Y and Z are each independently selected from the group consisting of carbon and nitrogen, whereas at least one of X, Y, and Z is nitrogen; and

R_1 - R_{10} are each independently selected from the group consisting of hydrogen, lone pair electrons, hydroxy, alkyl, cycloalkyl, phenyl, phenol, hydroxyphenol, dihydroxyphenol, aryl, alkenyl, alkynyl, heteroaryl, heteroalicyclic, halo, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, C-carboxy, O-carboxy, thiocarboxy, carbonyl, oxo, thiocarbonyl, sulfinyl, and sulfonyl, or absent, or, alternatively, at least two of R_1 - R_{10} form at least one five- or six-membered aromatic, heteroaromatic, alicyclic or heteroalicyclic ring,

and a pharmaceutically acceptable carrier.

59. The article-of-manufacture of claim 58, wherein:
each of X and Y is carbon; and
Z is nitrogen.

60. The article-of-manufacture of claim 59, wherein said double bond is between X and Y.

61. The article-of-manufacture of claim 59, wherein at least one of R_1 - R_{10} comprises an electronegative group.

62. The article-of-manufacture of claim 59 or 60, wherein at least one of R_1 and R_9 comprises an electronegative group.

63. The article-of-manufacture of any of claims 61 and 62, wherein said electronegative group is selected from the group consisting of hydroxy and halo.

64. The article-of-manufacture of claim 58, wherein at least one of R_1 - R_{10} comprises a hydroxy group.

65. The article-of-manufacture of claim 60, wherein at least one of R_1 - R_9 comprises a hydroxy group.

66. The article-of-manufacture of claim 65, wherein at least one of R_1 and R_9 is a hydroxy group.

67. The article-of-manufacture of claim 66, wherein each of R_2 - R_5 and R_7 is hydrogen and R_6 , R_8 and R_{10} are absent.

68. The article-of-manufacture of claim 67, wherein R_1 is hydrogen and R_9 is a hydroxy group.

69. The article-of-manufacture of claim 67, wherein R_1 is a hydroxy group and R_9 is hydrogen.

70. The article-of-manufacture of claim 65, wherein at least one of R_1 - R_{10} is a hydroxyalkyl.

71. The article-of-manufacture of claim 70, wherein at least one of R_7 and R_9 is a hydroxyalkyl.

72. The article-of-manufacture of claim 71, wherein each of R_1 - R_5 is hydrogen and R_6 , R_8 and R_{10} are absent.

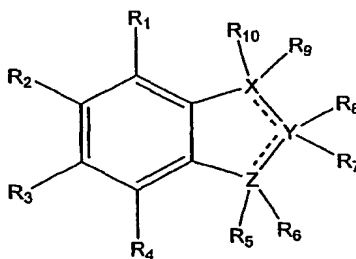
73. The article-of-manufacture of claim 71, wherein said hydroxyalkyl is hydroxymethyl.

74. The article-of-manufacture of claim 73, wherein R_7 is hydrogen and R_9 is said hydroxymethyl.

75. The article-of-manufacture of claim 74, wherein each of R_1 - R_5 is hydrogen and R_6 , R_8 and R_{10} are absent.

76. The article-of-manufacture of claim 71, wherein each of R_7 and R_9 is a hydroxyalkyl.

77. A compound having the general formula:



a pharmaceutically acceptable salt thereof, or a prodrug thereof,
wherein:

the dashed line denotes a double bond either between X and Y , or, between Y and Z ;

X , Y and Z are each independently selected from the group consisting of carbon and nitrogen, whereas at least one of X , Y , and Z is nitrogen; and

50

R_1 - R_{10} are each independently selected from the group consisting of hydrogen, lone pair electrons, hydroxy, alkyl, cycloalkyl, phenyl, phenol, hydroxyphenol, dihydroxyphenol, aryl, alkenyl, alkynyl, heteroaryl, heteroalicyclic, halo, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, C-carboxy, O-carboxy, thiocarboxy, carbonyl, oxo, thiocarbonyl, sulfinyl, and sulfonyl, or absent, or, alternatively, at least two of R_1 - R_{10} form at least one five- or six-membered aromatic, heteroaromatic, alicyclic or heteroalicyclic ring,

with the proviso that the compound is not any one selected from the group consisting of indole-3-carbynol, Tryptamine, 5-nitroindole, 7-methylindole, 5-methylindole, 3-methylindole, indole-3-butyric acid, indole-3-acetic acid, indole-6-carboxylic acid, indole-5-carboxylic acid, indole-3-carboxylic acid, indole-2-carboxylic acid, 2-indolinone, 3-hydroxyindole, 4-hydroxyindole, 5-hydroxyindole, Fmoc-D-Trp, Boc-L-Trp-OH, CBZ-D-Tryptophanamide, CBZ-L-Tryptophanamide, L-5-hydroxytryptophan, D-Tryptophan, L-Tryptophan, indole-3-propionic acid indole-3-ethanol, indole, N α -Boc-D-Trp, N α -Boc-L-Trp, Fmoc-Trp-OH and a 2,3-hydroxyalkyl indole.

78. The compound of claim 77, wherein:
each of X and Y is carbon; and
Z is nitrogen.

79. The compound of claim 78, wherein said double bond is between X and Y.

80. The article-of-manufacture of claim 77, wherein at least one of R_1 - R_{10} comprises an electronegative group.

81. The article-of-manufacture of claim 77 or 79, wherein at least one of R_1 and R_9 comprises an electronegative group.

82. The article-of-manufacture of any of claims 80 and 81, wherein said electronegative group is selected from the group consisting of hydroxy and halo.

83. The compound of claim 77, wherein at least one of R_1 - R_{10} comprises a hydroxy group.

84. The compound of claim 83, wherein at least one of R_1 - R_{10} is a hydroxyalkyl.

Figure 1

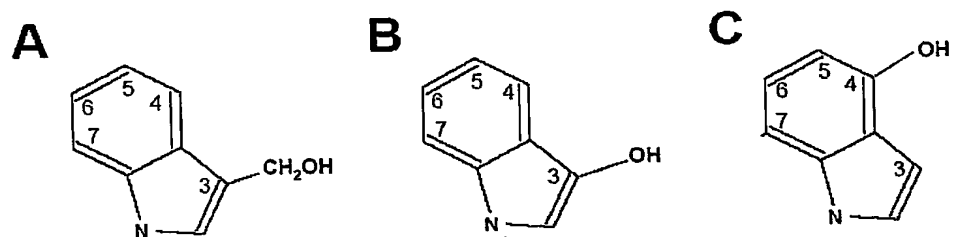


Figure 2

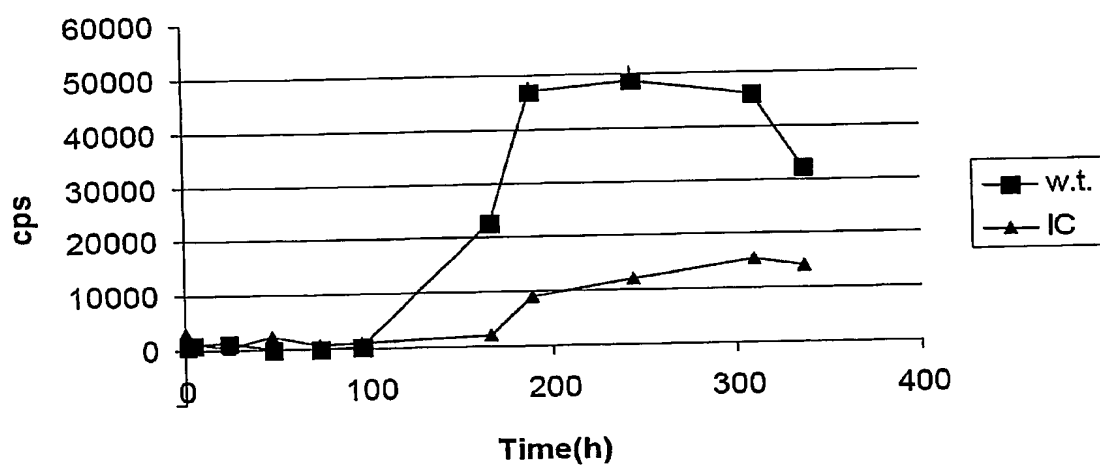


Figure 3a

Beta-Amyloid 1-40 5 μ M
192 h

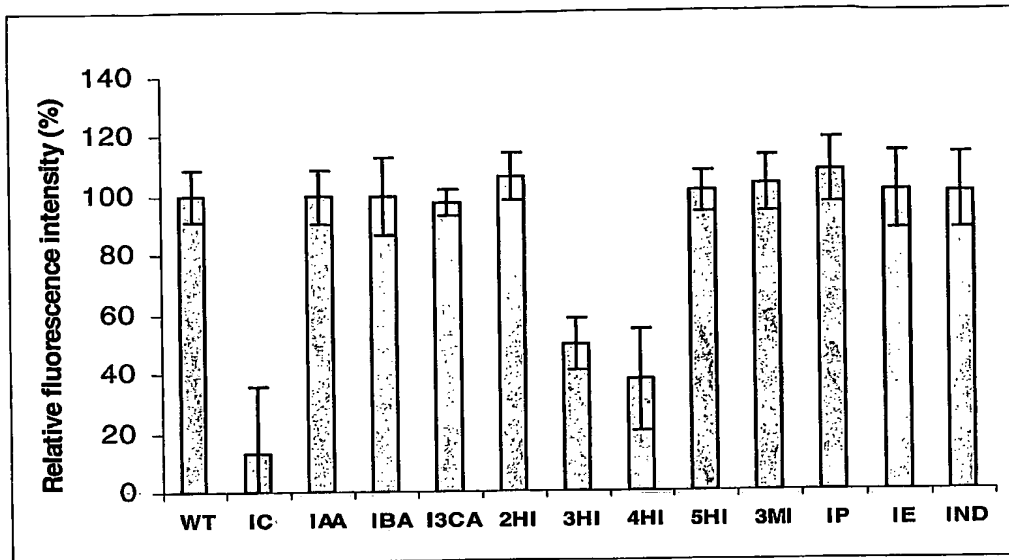


Figure 3b

Beta-Amyloid 1-40 5 μ M+IC 10 μ M
192 h

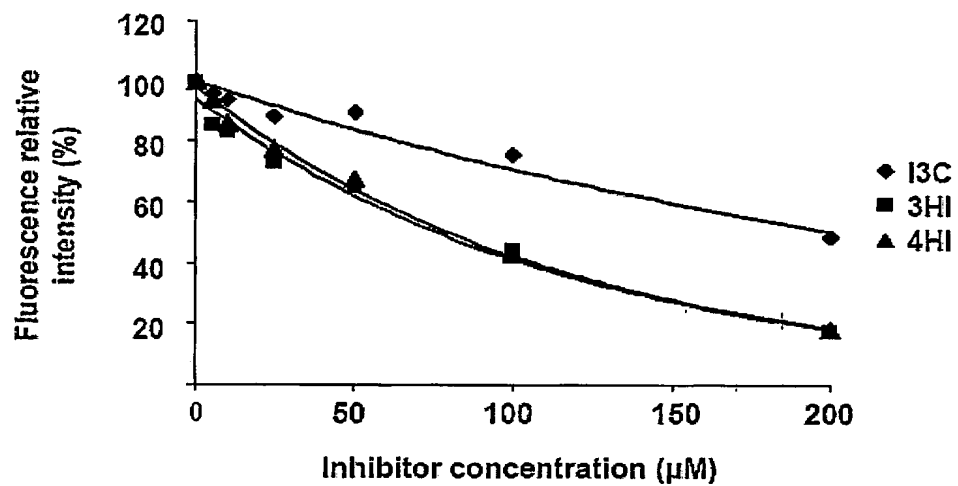


Figure 4



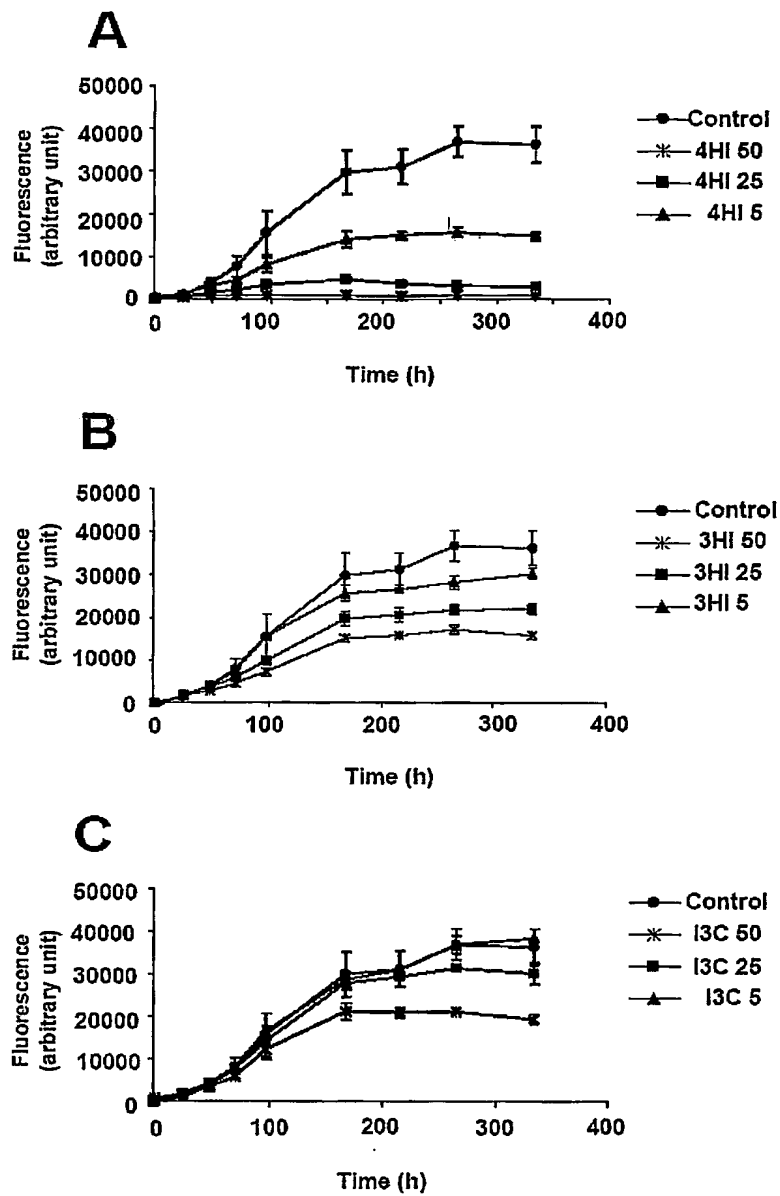
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Figure 5



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Figure 6



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Figure 7

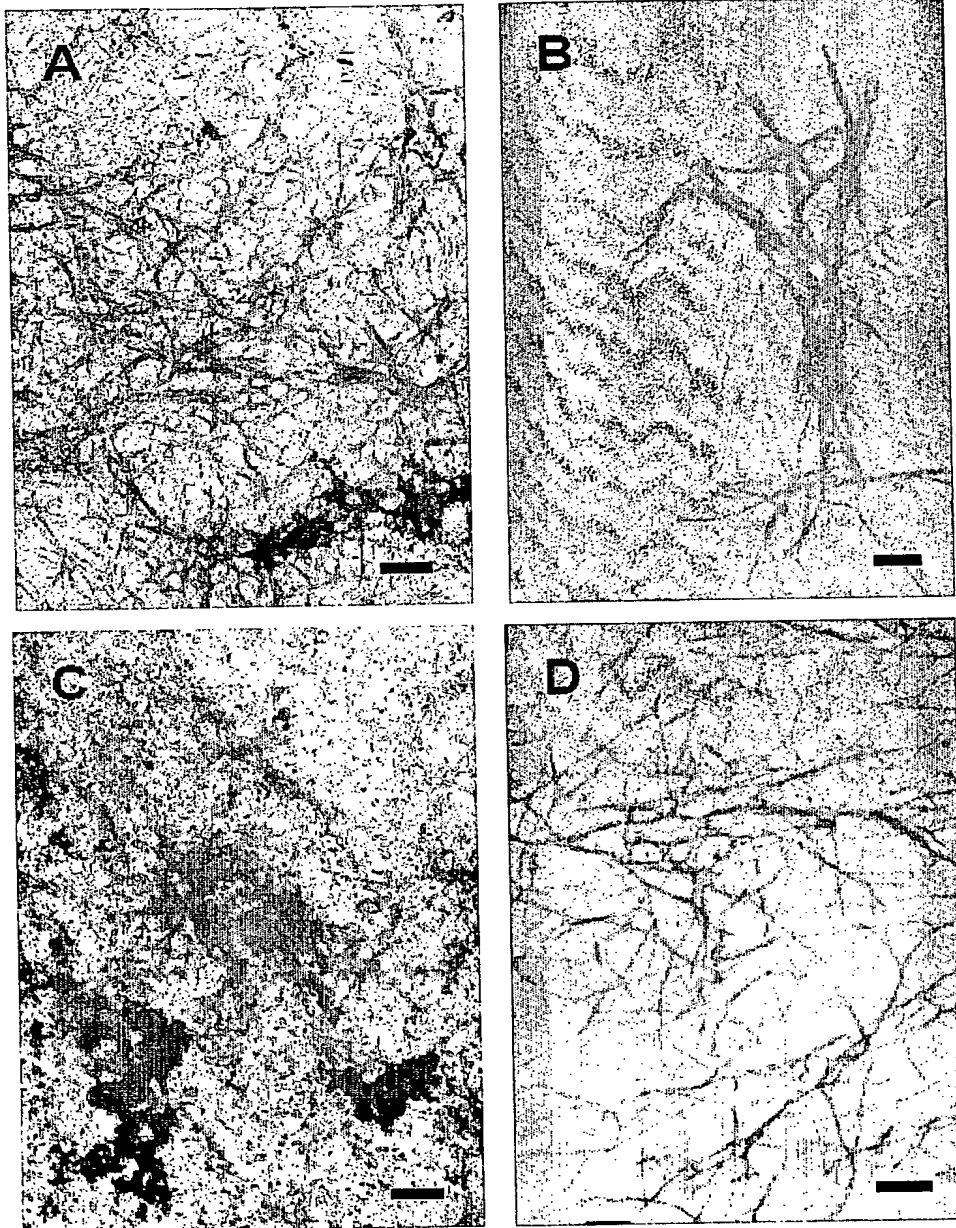
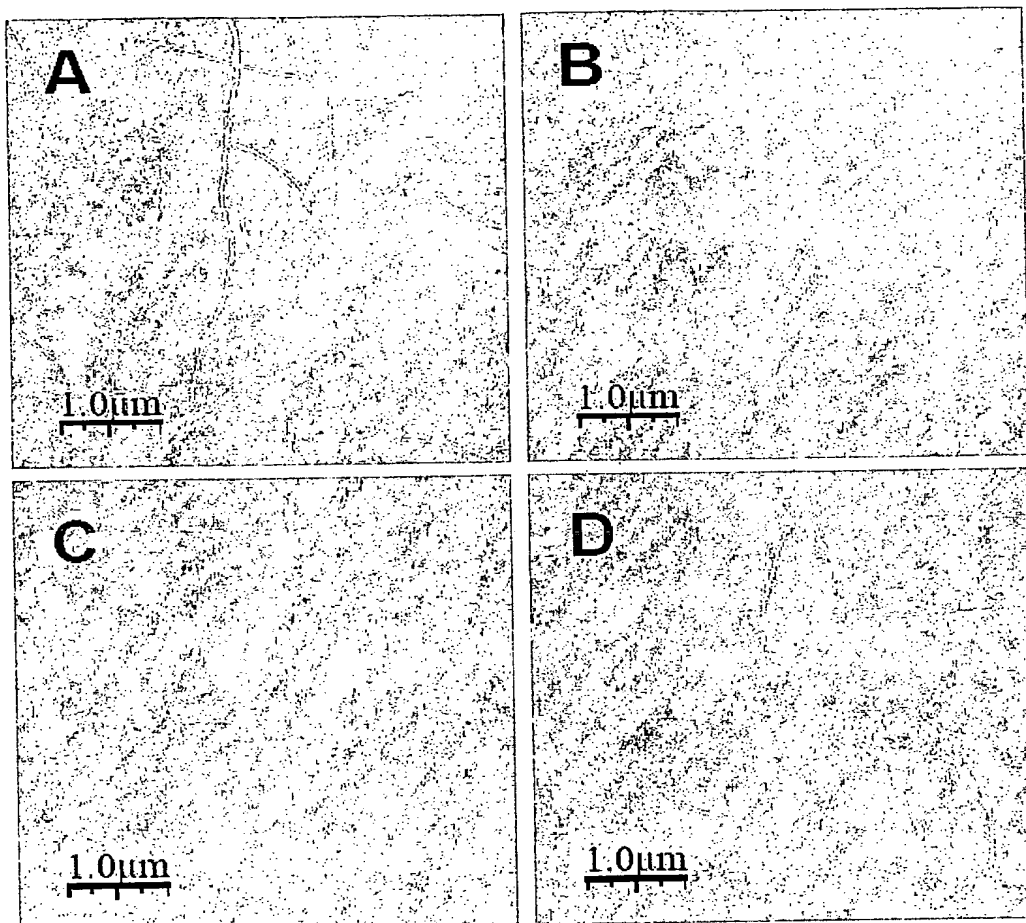
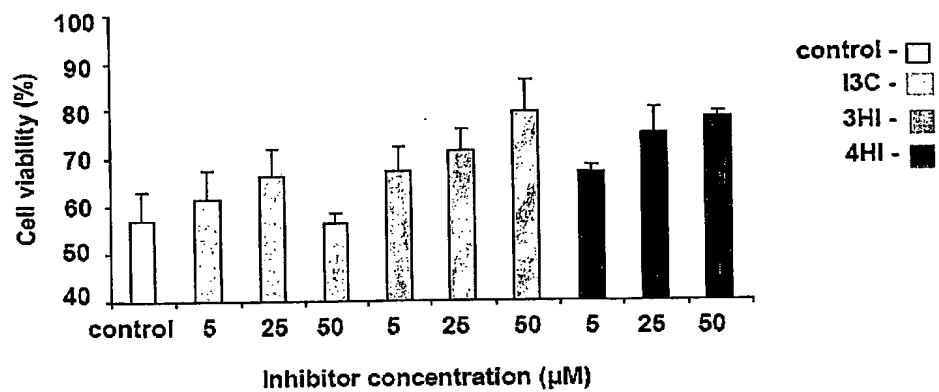
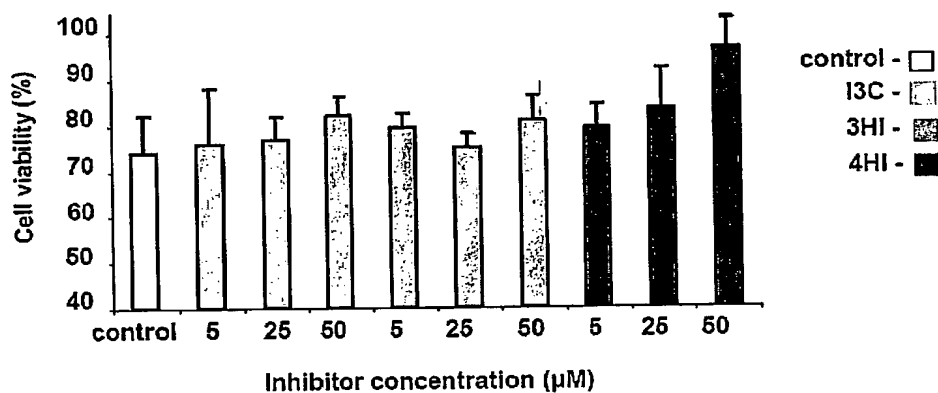


Figure 8



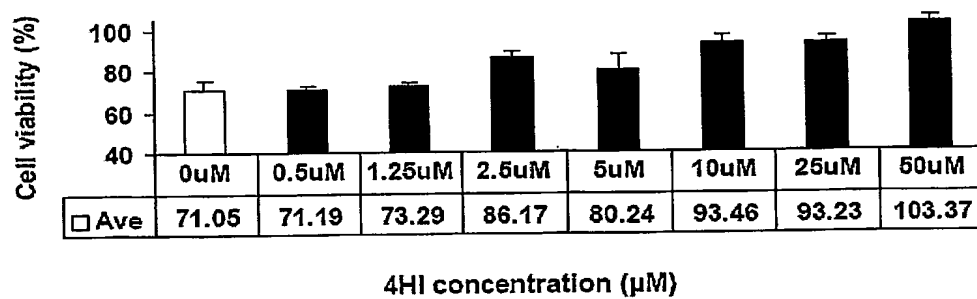
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Figure 9

A**B**

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Figure 10



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(74) Agent: **G. E. EHRLICH (1995) LTD.**; 11 Menachem Begin Street, 52 521 Ramat Gan (IL).

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(54) Title: COMPOSITIONS FOR TREATING AMYLOID ASSOCIATED DISEASES

(57) Abstract: Indole derivatives, compositions including same, and methods utilizing same for the treatment of amyloid associated diseases, such as type II diabetes mellitus, Alzheimer's dementia or diseases, systemic and localized amyloidosis, and prion-related encephalopathies are provided.

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2005/000902

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/403 A61K31/404 A61K31/415 A61K31/4164 C07D209/32
C07D209/12 A61P25/28 A61P5/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BEILSTEIN Data, CHEM ABS Data, WPI Data, PAJ, MEDLINE, BIOSIS, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	<p>US 2001/041732 A1 (GURLEY DAVID ET AL) 15 November 2001 (2001-11-15)</p> <p>pages 1-2, paragraph 21 page 2, paragraph 29 page 3, paragraph 39 claims 5,7,9,10</p> <p>----- -/~</p>	<p>1-4,6-8, 20-23, 25-27, 39-42, 44-46, 58-61, 63-65</p>

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	<p>NAKAJIMA, TERUO: "Amine precursor therapy: manipulation of brain amine activity with precursor amino acid" PSYCHIATRY AND CLINICAL NEUROSCIENCES, 51(5), 267-274 CODEN: PCNEFP; ISSN: 1323-1316, 1997, XP008064215</p> <p>page 269, column 1, paragraphs 2,3</p>	<p>1-4,6-8, 20-23, 25-27, 39-42, 44-46, 58-61, 63-65</p>
X	<p>wo 03/039540 A (SEPRACOR INC; HEEFNER, DONALD, L; CURRIE, MARK, G; ROSSI, JR., RICHARD) 15 May 2003 (2003-05-15)</p> <p>page 1, paragraph 2 page 6, paragraph 16 - page 7, paragraph 17 page 13, paragraph 41 page 13, paragraph 43 - page 14, paragraph 44 page 16, paragraph 47 claims 1,4,7,8,11</p>	<p>1-4,6-8, 20-23, 25-27, 39-42, 44-46, 58-61, 63-65, 77-80, 82,83</p>
X	<p>wo 03/070269 A (SCHRAERMAYER, ULRICH) 28 August 2003 (2003-08-28)</p> <p>page 1, paragraph 1 page 3, paragraph 5 page 6, paragraph 3 claim 2</p>	<p>1-4,6-8, 20-23, 25-27, 39-42, 44-46, 58-61, 63-65, 77-80, 82,83</p>
X	<p>EP 0 421 946 A (POLIFARMA S.P.A) 10 April 1991 (1991-04-10)</p> <p>page 2, lines 1-15,44-46 examples 4,5 page 8, lines 39,40 claims 6-8</p>	<p>1-10, 20-29, 39-48, 58-67, 77-83</p>

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2005/000902

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	<p>wo 99/42102 A (SOUTH ALABAMA MEDICAL SCIENCE FOUNDATION; NEW YORK UNIVERSITY) 26 August 1999 (1999-08-26)</p> <p>page 4, lines 8-18 page B, lines 18-21 page 5, line 31 - page 6, line 8 page 6, line 24 - page 7, line 12 page 10, line 24 - page 11, line 27 examples 1,6 claims 1,9,11,12,19-24</p> <p style="text-align: center;">-----</p>	<p>1-5 , 20-24 , 39-43 , 58-62</p>
X	<p>US 2004/029830 AI (HEBERT ROLLAND F) 12 February 2004 (2004-02-12)</p> <p>page 1, paragraphs 3,12 pages 1-2, paragraph 13 page 2, paragraphs 15,23 page 2, paragraph 24 - page 3, paragraph 25 claims 1,4-7,10,12,13</p> <p style="text-align: center;">-----</p>	<p>1-5 , 20-24 , 39-43 , 58-62</p>
X	<p>LEE, MIN WON ET AL: "Anti- diabetic constituent from the node of lotus rhizome (Nelumbo nucifera Gaertn.)" NATURAL PRODUCT SCIENCES, 7(4), 107-109 CODEN: NPSCFB; ISSN: 1226-3907, 2001, XP001154675 abstract page 108, column 1, last paragraph - column 2, paragraph 1</p> <p style="text-align: center;">-----</p>	<p>1-5 , 20-24 , 39-43 , 58-62</p>
X	<p>wo 03/024443 A (UNIVERSITY OF FLORIDA; MARTYNYUK, ANATOLY, E; DENNIS, DONN, MICHAEL; G) 27 March 2003 (2003-03-27)</p> <p>page 3, paragraph 10 page 4, paragraph 12 pages 4-5, paragraph 14 pages 19-20, paragraph 73 claims 1-3,14,16,48,51,53,66,69</p> <p style="text-align: center;">-----</p>	<p>1-5 , 20-24 , 39-43 , 58-62</p>
X	<p>wo 00/24390 A (THE UNIVERSITY OF BRITISH COLUMBIA; REINER, PETER, B; LAM, FRED, CHIU-) 4 May 2000 (2000-05-04)</p> <p>page 8, line 29 - page 9, line 6 page 21, lines 13-17 page 23, lines 1-7 page 24, line 16 - page 25, line 2 claims 1,2,11,12,15-17,20,21,24,29,30,53,54,63,64,67,68</p> <p style="text-align: center;">-----</p> <p style="text-align: center;">-/-</p>	<p>1-5 , 20-24 , 39-43 , 58-62</p>

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International application No

PCT/IL2005/000902

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Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	<p>DATABASE WPI Secti on Ch , Week 200039 Derwent Publ icati ons Ltd . , London , GB ; Cl ass B05 , AN 2000-451668 XP002382814 HONMA T. ET AL: "Use of a thromboxane A2 antagoni st or synthase inhibi tor for treating central nervous system di seases , e . . g. Al zheimer type dementi a ." & wo 00/30683 AI (SHIONOGI & co LTD) 2 June 2000 (2000-06-02) abstract</p>	<p>1-4 ,6, 20-23, 25, 39-42, 44 , 58-61 , 63 , 77-80 ,82</p>
X	<p>US 2004/152672 AI (CARSON DENNIS A ET AL) 5 August 2004 (2004-08-05)</p> <p>page 1, paragraph 10 - page 2, paragraph 11 page 2, paragraph 14 page 5, paragraph 70 cl aims 1,3,9</p>	<p>1-3 , 20-22 , 39-41 , 58-60 , 77-79</p>
X	<p>LOSERT W ET AL: "Effects of indol e 3 al kanecarboxyl ic aci ds on glucose uti lizati on in rats" ARZNEIMITTEL-FORSCHUNG/DRUG RESEARCH 1975 , vol . 25, no. 6, 1975 , pages 880-887 , XP008064216 page 880 , col umn 1, paragraph 6 page 886 , col umn 2, paragraphs 4,5 page 887 , col umn 1, paragraph 3</p>	<p>1-5 , 20-24 , 39-43 , 58-62 , 77-81</p>
X	<p>DATABASE WPI Secti on Ch , Week 200328 Derwent Publ icati ons Ltd . , London , GB ; Cl ass B02 , AN 2003-286683 XP002382815 "Pharmaceutical compositi on for prophyl axi s and treatment of uterus cervi x dyspl asi a and cancer and larynx papi llomatosi s and methods of prophyl axi s and treatment of si ad sicknesses based on thereof. " & RU 2 196 568 CI (KISELEV V I) 20 January 2003 (2003-01-20) abstract</p>	<p>39-46, 51-56, 58-65 , 70-75</p>

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INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2005/000902

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	<p>KAZUMI KON-YA ET AL: "INDOLE DERIVATIVES AS POTENT INHIBITORS OF LARVAL SETTLEMENT BY THE BARNACLE, BALANUS AMPHITRITE" BIOSCIENCE BIOTECHNOLOGY BIOCHEMISTRY, JAPAN SOC. FOR BIOSCIENCE, BIOTECHNOLOGY AND AGROCHEM, TOKYO, JP, vol. 58, no. 12, 1994, pages 2178-2181, XP008064229 ISSN: 0916-8451 compound 102</p> <p style="text-align: center;">-----</p>	77-84
X	<p>P. APPUKKUTTAN, E. VAN DER EYCKEN, W. DEHAEN: "Microwave enhanced formation of electron rich aryl boronates" SYNLETT, no. 8, 2003, pages 1204-1206, XP002382809 figures scheme, 4; compounds 5A, 5B, 5C, 5D</p> <p style="text-align: center;">-----</p>	77-83
X	<p>US 3 042 685 A (ROUSSEL UCLAF) 3 July 1962 (1962-07-03) examples 3, 4, 6, 7</p> <p style="text-align: center;">-----</p>	77-80, 82
X	<p>DATABASE CROSSFIRE BEILSTEIN [Online] Beilstein Institut zur Förderung der Chemischen Wissenschaften, Frankfurt am Main, DE; XP002382811 Database accession no. 116671 (BRN) compounds INDOL-2-YL-METHANOL & BEUGELMANS R.: BULL. SOC. CHIM. FR., 1969, pages 335-336,</p> <p style="text-align: center;">-----</p>	77-80, 82-84
X	<p>US 2 920 080 A (LES LABORATOIRES FRANCAIS DE CHIMIOThERAPIE) 5 January 1960 (1960-01-05) column 2, lines 31-45</p> <p style="text-align: center;">-----</p>	77-80, 82
X	<p>US 3 625 973 A (MARC JULIA) 7 December 1971 (1971-12-07) column 1, lines 4-8 examples 4-6</p> <p style="text-align: center;">-----</p>	77-80, 82, 83
X	<p>FR 1 373 316 A (SANDOZ S. A) 25 September 1964 (1964-09-25) page 2, column 2, line 40 page 3, column 1, lines 18, 55, 56 page 3, column 2, lines 28, 29</p> <p style="text-align: center;">-----</p>	77-84
X	<p>US 3 790 596 A (SHKILKOVA V, SU ET AL) 5 February 1974 (1974-02-05) examples 3-5, 7-9</p> <p style="text-align: center;">-----</p>	77-80, 82
	-/--	

INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2005/000902

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	US 3 976 639 A (BATCHO ET AL) 24 August 1976 (1976-08-24) exampl es 20, 22, 24, 26, 29, 35, 37, 44, 47, 51, 53, 55 -----	77-80 ,82
P,X	wo 2005/016339 A (CASE WESTERN RESERVE UNIVERSITY ; LANDRETH, GARY ; COMBS, COLIN ; HIRSCH,) 24 February 2005 (2005-02-24) page 1, lines 5-9 page 2, lines 22-29 page 3, lines 28-32 page 7, lines 6-17 page 37, lines 21-23 page 38; compound XVII page 40, lines 19-33 page 52, lines 17-19 exampl e 6 cl aims 1,4 -----	1-4 , 20-23 , 39-42 , 58-61 , 77-80
E	wo 2005/085867 A (PFIZER JAPAN, INC ; TANIGUCHI, YASUHIRO ; KACHI, HIROKO ; TOMOTOSHI, KIMI) 15 September 2005 (2005-09-15) page 9, lines 3-24 page 20, lines 5-13 cl aims 21,23 -----	1-8 , 20-27 , 39-46 , 58-65 , 77-83
E	wo 2006/020681 A (HYGEIA BIOLOGICAL SYSTEMS ; BANERJEE, AMIT) 23 February 2006 (2006-02-23) page 2, lines 14-17 page 4, lines 6-19 page 10, lines 8-12 page 22, lines 11-30 page 36, line 30 - page 37, line 2 cl aims 45,47 -----	1-11 , 20-30 , 39-49 , 58-68
T	COHEN T. ET AL.: "Inhibiti on of amyloid fibri l formati on and cytotoxi city by hydroxyi ndol e deri vati ves" BIOCHEMISTRY, vol . 45, 2006 , pages 4727-4735 , XP002382810 abstract page 4728, col umn 1, last paragraph page 4728, col umn 2, paragraph 2 figure 1 page 4729, col umn 1, paragraph 4 page 4732, col umn 2, paragraphs 2, 3 page 4733, col umn 2, paragraph 4 -----	1-84

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Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons

- 1 ☒ Claims Nos
because they relate to subject matter not required to be searched by this Authority, namely

Although claims 1-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
- 2 ☐ Claims Nos
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically
- 3 ☐ Claims Nos
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6 4(a)

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows

- 1 ☐ As all required additional search fees were timely paid by the applicant this International Search Report covers all
searchable claims
- 2 ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee
- 3 ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos
- 4 ☐ No required additional search fees were timely paid by the applicant. Consequently this International Search Report is restricted to the invention first mentioned in the claims, it is covered by claims Nos

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest
- ☐ No protest accompanied the payment of additional search fees

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IL2005/000902

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
US 2001041732	AI	15-11-2001	AT	249827	T	15-10-2003
			AU	770849	B2	04-03-2004
			AU	4302399	A	23-11-1999
			BR	9910180	A	09-01-2001
			CA	2331070	AI	11-11-1999
			CN	1299279	A	13-06-2001
			CN	1637149	A	13-07-2005
			DE	69911400	DI	23-10-2003
			DE	6991 1400	T2	08-07-2004
			EE	200000640	A	15-04-2002
			EP	1079828	AI	07-03-2001
			HK	1034205	AI	21-01-2004
			HU	.0102504	A2	28-1 1-2001
			ID	27289	A	22-03-2001
			JP	2002513757	T	14-05-2002
			NO	20005503	A	04-01-2001
			PL	343923	AI	10-09-2001
			RU	2225203	C2	10-03-2004
			WO	9956745	AI	11-11-1999
			SK	15702000	A3	06-08-2001
			TR	200003244	T2	21-03-2001
			TW	542718	B	21-07-2003
			UA	65617	C2	15-03-2001
			US	6277870	BI	21-08-2001
			ZA	200006133	A	30-01-2002
<hr/>						
WO 03039540	A	15-05-2003	NONE			
<hr/>						
WO 03070269	A	28-08-2003	AU	2003218870	AI	09-09-2003
			DE	10206723	AI	04-09-2003
			DE	10390601	D2	27-01-2005
			EP	1478389	AI	24-11-2004
			US	2005142128	AI	30-06-2005
<hr/>						
EP 0421946	A	10-04-1991	DE	69029186	DI	02-01-1997
			DE	69029186	T2	27-03-1997
			IT	1237472	B	07-06-1993
			US	5210215	A	11-05-1993
<hr/>						
WO 9942102	A	26-08-1999	AU	757925	B2	13-03-2003
			AU	2782899	A	06-09-1999
			BR	9908165	A	31-10-2000
			CA	2321455	AI	26-08-1999
			CN	1292693	A	25-04-2001
			EA	5244	BI	30-12-2004
			EP	1056452	AI	06-12-2000
			HK	1036588	AI	25-02-2005
			JP	2002503691	T	05-02-2002
			MX	PA00008216	A	22-11-2004
			NZ	506260	A	30-05-2003
<hr/>						
US 2004029830	AI	12-02-2004	NONE			
<hr/>						
WO 03024443	A	27-03-2003	EP	1446111	AI	18-08-2004
			US	2003216472	AI	20-11-2003
			US	2004266851	AI	30-12-2004
			US	2004147590	AI	29-07-2004
			US	2003055099	AI	20-03-2003

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IL2005/000902

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0024390	A	04-05-2000	AU 762593 B2	26-06-2003
			AU 1112800 A	15-05-2000
			CA 2348019 A1	04-05-2000
			EP 1123090 A1	16-08-2001
			JP 2002528411 T	03-09-2002
WO 0030683	A1	02-06-2000	AU 1179000 A	13-06-2000
US 2004152672	A1	05-08-2004	AU 8322401 A	18-02-2002
			CA 2419060 A1	14-02-2002
			EP 1307459 A2	07-05-2003
			JP 2004518615 T	24-06-2004
			WO 0212188 A2	14-02-2002
RU 2196568	C1	20-01-2003	NONE	
US 3042685	A		NONE	
US 2920080	A		NONE	
US 3625973	A	07-12-1971	CH 498112 A	31-10-1970
			DE 1795147 A1	30-12-1971
			FR 1540484 A	27-09-1968
			GB 1240013 A	21-07-1971
FR 1373316	A	25-09-1964	BE 638366 A	
US 3790596	A	05-02-1974	NONE	
US 3976639	A	24-08-1976	NONE	
WO 2005016339	A	24-02-2005	AU 2003254146 A1	07-03-2005
WO 2005085867	A	15-09-2005	NONE	
WO 2006020681	A	23-02-2006	US 2006035890 A1	16-02-2006